ENDOCRINOLOGY

VOLUME 60 JUNE, 1957 NUMBER 6

Copyright © 1957 by the Endocrine Society

A NEW ASSAY FOR THYROTROPIC HORMONE BASED UPON THYROIDAL PHOSPHOLIPID TURNOVER *IN VITRO*[1]

WARNER H. FLORSHEIM, NATHAN MOSKOWITZ, JOSEPH R. SCHWARTZ[2] AND M. E. MORTON[3]

Radioisotope Service, U. S. Veterans Administration Hospital, Long Beach, California and the Department of Physiological Chemistry, University of California Medical School, Los Angeles, California

THE assay of protein hormones still presents many problems. Most biological assay procedures are slow and inaccurate and require large numbers of animals. *In vitro* tests using tissues obtainable from the slaughterhouse therefore offer considerable advantages, especially to laboratories where the need for hormones assays arises only occasionally. To date, only a single *in vitro* assay for thyrotropin has been proposed (1) and the specificity and precision of this method have not been explored. We therefore considered it of interest to investigate further an *in vitro* effect of thyrotropin which was first described from this laboratory (2). This publication presents our findings regarding the sensitivity, specificity and limitations of the stimulation of radiophosphorus incorporation into the phospholipids of surviving thyroid slices by pituitary thyrotropic hormone.

METHODS

Steer or heifer thyroids were obtained at the slaughterhouse and used within two hours after sacrifice.[4] The glands were freed of fat and connective tissue and cut into

Received March 31, 1956.

[1] Aided in part by a grant from the National Heart Institute (H-2013) of the National Institutes of Health, U. S. Department of Health, Education and Welfare.

Presented at the 1956 meeting of the Endocrine Society.

[2] Present address: Department of Chemistry, Loyola University, Playa del Rey, California.

[3] Present address: 6515 Wilshire Blvd., Los Angeles, California.

[4] We wish to thank Dr. Jager and Mr. Baxter of the Alpha Beta Packing House, Wintersburg, California for making steer thyroids available to us.

slices about 0.3 mm. thick. The slices were washed with ice cold saline and blotted on hardened filter paper. Three hundred mg. of slices were weighed with a torsion balance and added to 20 cc. beakers containing 3.0 cc. of Krebs-Ringer bicarbonate buffer of pH 7.4 and 1.0 μC of radioactive inorganic phosphate.[5] The thyrotropin preparation to be assayed was then added dissolved in 0.20 cc. of buffer. Control samples received 0.20 cc. of buffer only. The samples were incubated on a Dubnoff shaker for 3 hours at 37° C in an atmosphere of oxygen containing 5% carbon dioxide.

After the incubation the contents of the beakers were transferred quantitatively to test tubes and treated with an equal volume of 12% TCA. The precipitate was washed three times with 6% TCA to free it of most of the inorganic and acid soluble phosphorus. The lipids were extracted with four changes of methylal containing 10% methanol. The combined lipid extracts were evaporated to dryness at 50° C with a stream of nitrogen and the residue transferred to planchets with chloroform. For the fractionation of the phospholipid fraction into its alkali-stable ("sphingomyelin") and alkali-labile components the method of Petersen (3) was used.

In experiments testing the effects of substances insoluble in buffer, these were added to the medium dissolved in a minimum volume of propylene glycol, which was found not to affect the phospholipid labelling process.

RESULTS

Under the conditions specified, 300 mg. of fresh thyroid slices incorporated approximately 0.33% of the phosphate-P^{32} in the medium into the phospholipid fraction. Under the influence of added thyrotropin up to 240% of this amount was incorporated. The total phosphorus content of the lipid fraction remained unchanged.

The thyroidal P^{32} incorporation into the lipid fraction varied with the logarithm of the thyrotropin concentration, as shown in Figure 1. The linear range of the assay procedure appeared to be from 5 to 200 milliunits of TSH (using U.S.P. Reference Standards or Armour "Thytropar"). By incubating 100 mg. of thyroid slices in 1.0 cc. of medium, as little as 2 milliunits of thyrotropin could be determined. Using the statistical methods of Emmens (4) the index of precision "λ" was calculated. It proved to be between 0.20 and 0.24 in assays carried out during the spring and fall months. However, during hot summer weather the responsiveness of thyroid tissue decreased and the slope of the dose-response curve flattened appreciably. Thus during September values of λ as high as 0.31 were obtained.

While thyroid tissue retained its ability to synthesize phospholipids and to respond to thyrotropin for many hours at 5° C, freezing and thawing eliminated phospholipid synthesis almost completely. Tissue preserved by the method of Parkes and Smith (5) for maintaining endocrine tissues suitable for transplantation was only 3% as active as non-frozen control thyroid.

It has been shown previously (2) that the incorporation of P^{32} into the

[5] The radiophosphorus was obtained from the Oak Ridge National Laboratory, of the U. S. Atomic Energy Commission.

lipids of rat and beef kidney and liver is not influenced by thyrotropin. This also proved to be true for beef submaxillary gland tissue. However, stimulation was observed in 14 out of 16 samples of pathological human thyroid tissue obtained at surgery. Stimulation was observed in samples of 2 carcinomas, 4 out of 5 adenomas, 3 out of 4 nodular goiters, 6 colloid goiters, and one case of chronic thyroiditis.

Thyrotropin affected the synthesis of phospholipids only. No effect upon

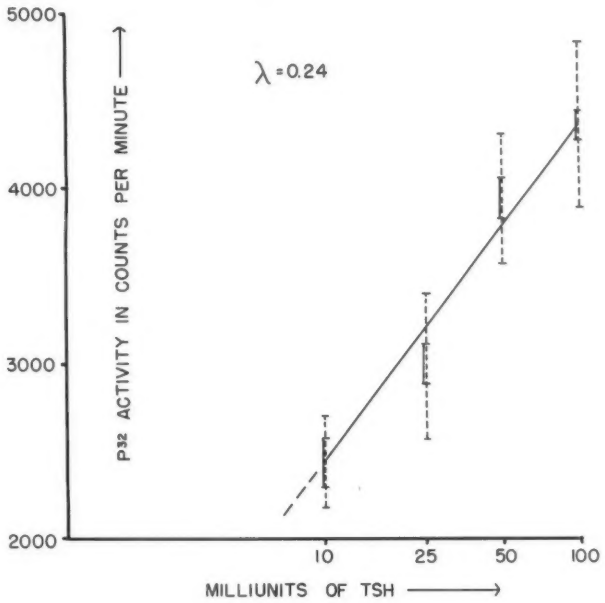


FIG. 1. Typical dose response curve obtained with Parke-Davis "50P_4" thyrotropin.
Solid Vertical Lines: standard errors
Dashed Vertical Lines: standard deviations

either the acid soluble or the protein phosphorus fractions could be demonstrated. Within the phospholipids the effect of thyrotropin also was found to be selective: the labelling of the alkali-labile phospholipids increased, while spingomyelin was entirely unaffected.

Fractionation of the phospholipids by Dawson's (6) procedure showed that the stimulation was not due predominantly to an increased synthesis of phosphatidic acids as has been observed in stimulated pancreas slices by Hokin (7).

In order to define the specificity of the phospholipid labelling of thyroid slices as an assay procedure, we studied the effects of other hormones upon radiophosphorus incorporation. In Table 1 are shown the effects of 7 hormones of extra-pituitary origin. With the exception of chorionic gonado-

TABLE 1. EFFECT OF EXTRA-PITUITARY HORMONES ON P^{32} INCORPORATION INTO THE PHOSPHOLIPIDS OF SURVIVING THYROID SLICES

CompoundQtested	TSH activity found
Cortisone	0 mU./mg.
Cortisone acetate	0 mU./mg.*
Desoxycorticosterone glycoside	0 mU./mg.*
Testosterone	0 mU./mg.
Insulin (Iletin-Lilly)	0 mU./mg.
Chorionic Gonadotropin	12 mU./100 I.U.
Thyroxine	0 mU./γ
Estrogen ("Premarin"-Ayerst)	0 mU./mg.*

* Preparation depressed P^{32} incorporation below control levels.

tropin, none stimulated phospholipid incorporation detectably, although several of the preparations proved to be toxic at the levels employed and lowered the P^{32} incorporation into all tissue fractions. The effect of chorionic gonadotropins is not surprising since van Wagenen and Simpson (8) found significant levels of thyrotropin in pregnancy urines.

In Table 2 are summarized our results with anterior pituitary hormones.[6] ACTH was found to be inactive. Growth hormone also was shown to be

TABLE 2. EFFECT OF ANTERIOR PITUITARY HORMONES ON P^{32} INCORPORATION INTO THE PHOSPHOLIPIDS OF SURVIVING THYROID SLICES

Compounds tested	TSH activity found
Corticotropin "ACTHAR"*	2 mU./mg.
Growth Hormone 264-159 A*	45 mU./mg.
Growth Hormone R377237*	68 mU./mg.
Growth Hormone 359-265*	0 mU./mg.
Follicle Stimulating Hormone 151X*	11 mU./mg.
Follicle Stimulating Hormone 264-151X*	26 mU./mg.
Luteinizing Hormone 22780*	124 mU/mg.
Pituitary Gonadotropin (Searle)	4 mU./R.U.
Pituitary Gonadotropin (Squibb 1949)	48 mU./R.U.
Pituitary Gonadotropin (Squibb 1954)	2 mU./R.U.

* Product of Armour Laboratories.

inactive, although in early experiments thyrotropin contamination in the impure preparations then available gave misleading results. A sample of follicle-stimulating hormone had an effect equivalent to only half of the stated maximal thyrotropin contamination and thus was assumed to be inactive. A very crude sample of luteinzing hormone was definitely active, but it is doubtful whether the activity was due to luteinizing hormone since the three "pituitary gonadotropin" preparations tested had stimulating effects which were completely unrelated to the gonadotropic potency of the samples. Thus a preparation purchased from Squibb in 1948 had about 12 times the potency per unit gonadotropic activity of a Searles preparation,

[6] We are grateful to Dr. S. Steelman of the Armour Laboratories for samples of highly purified Growth Hormone and Follicle Stimulating Hormone.

while the latter was twice as potent as the material currently supplied by Squibb.

The effect of thiouracil and thiocyanate upon tyroidal phospholipid labelling was shown to be negligible by Schwartz and Morton (9). Propylthiouracil also did not interfere with either the control lipid labelling or the thyrotropin stimulation, and neither did iodide in concentrations up to 0.12 *M*.

DISCUSSION

As an assay procedure our test based on the *in vitro* stimulation of thyroidal phospholipid labelling by thyrotropic hormone is less sensitive than the animal assays of Del Conte and Stux (10), Greenspan *et al.* (11, 12) or D'Angelo (13). Gilliland and Strudwick (14), basing their procedure upon the discharge of radioiodine from the chick thyroid caused by thyrotropin, report a similar sensitivity and interference by gonadotropins. In our hands the Gilliland and Strudwick assay, modified by the addition of propylthiouracil to the diet in order to increase the slope of the dose-response curve, gave a λ of 0.22 and a usable range of 3 to 50 milliunits of TSH. Piotrowski and coworkers (15), using a much more difficult procedure, report a sensitivity similar to that of our assay, a lower reliability, and definite interference by follicle-stimulating-hormone. The rat assay of Levey, Cheever and Roberts (16) appears superior to ours in both precision and sensitivity, but it employs an expensive test animal. The method of Greenspan *et al.*, while sensitive and convenient, has the rather high λ of 0.34 and therefore requires far more determinations for an equal accuracy.

Since our assay procedure obviates the need for a large animal colony, can be completed within two working days and is exceedingly insensitive to goitrogens, iodide and thyroxine, we feel that it offers definite advantages over most other methods. Its cheapness and good precision also make it attractive.

No comprehensive study has ever been made to relate the various effects of thyrotropin and the assay procedures based upon them to each other. We have carried out a comprehensive study of the interrelation of our assay with the *in vitro* assay of Hilliard and West (17) which is based upon the formation of an enzymatically inactive complex between pepsin and thyrotropin. Agreement was excellent in a large number of purified TSH preparations with only one exception. A sample of a "partial thyrotropin" extracted from human urine by Dr. F. S. Greenspan (12) which had been shown to be active in the chick assay of Greenspan *et al.* (11) but proved inactive in increasing the height of thyroidal epithelial cells, proved to be the sole exception. It was as active in the Hilliard-West assay as in Greenspan's, but proved to be completely inactive in the phospholipid-stimulation assay. In limited series of assays there was good agreement between

our assay and those of Greenspan and of Gilliland and Strudwick, but these results were qualitative only and not extensive enough for statistical evaluation. Our assay also appears to agree well with that of Piotrowski *et al.* (15), since we have confirmed repeatedly the relative potencies of commerical Armour thyrotropin preparations which where calibrated by means of this assay.

SUMMARY

An assay procedure based on the incorporation of radiophosphorus into the lipid fraction of surviving beef thyroid glands *in vitro* has been devised. The usable range of this assay is from 2 to 200 milliunits of thyrotropin and the index of precision (λ) is 0.22. The stimulation of phospholipid turnover by thyrotropin appears to be limited to thyroid tissue. Interference by other hormones has been investigated.

REFERENCES

1. Bakke, J. L. and N. Lawrence, *Am. J. Med.* **17**: 116. 1954.
2. Morton, M. E. and J. R. Schwartz: *Science* **117**: 103. 1953.
3. Petersen, V. P.: *Scand. J. Lab. & Clin. Invest.* **2**: 14. 1950.
4. Emmens, C. W.: Principles of Biological Assay, Chapman and Hall, London, 1948.
5. Parkes, A. S. and A. U. Smith: *Proc. Roy. Soc. (London)* **B140**: 455. 1953.
6. Dawson, R. M. C.: *Biochim. et Biophys. Acta* **41**: 374. 1954.
7. Hokin, L. E. and M. R. Hokin: *Canad. J. Biochem. & Physiol.* **34**: 349. 1956.
8. Van Wagenen, G. and M. E. Simpson: *Proc. Soc. Exp. Biol. & Med.* **90**: 346. 1955.
9. Schwartz, J. R. and M. E. Morton: *Proc. Soc. Exp. Biol. & Med.* **88**: 50. 1955.
10. Del Conte, E. and M. Stux: *Ciencia e Invest.* **9**: 524. 1953.
11. Greenspan, F. S., J. P. Kriss, L. E. Moses and W. Lew: *Endocrinology* **58**: 767. 1956.
12. Greenspan, F. S., L. E. Moses, J. P. Kriss and W. Lew: *Clin. Res. Proc.* **4**: 60. 1956.
13. D'Angelo, S. A., A. S. Gordon and H. A. Charipper: *Endocrinology* **31**: 217. 1942.
14. Gilliland, I. C. and J. I. Strudwick: *Clin. Sci.* **12**: 265. 1953.
15. Piotrowski, L. J., S. L. Steelman and F. C. Koch: *Endocrinology* **52**: 489. 1953.
16. Levey, H. A., E. Cheever and S. Roberts: *Endocrinology* **58**: 420. 1956.
17. Hilliard, J. and P. M. West: *Endocrinology* in press.

SELECTIVITY OF THE EFFECTS OF HYPOTHALAMIC LESIONS ON PITUITARY TROPHIC HORMONE SECRETION IN THE RAT[1]

E. M. BOGDANOVE

Department of Anatomy, Albany Medical College of Union University, Albany, New York

IN THE rat, hypothalamic lesions may result in testicular atrophy (1, 2, 3), disturbances of the estrus cycle (4, 5), and "goiter block" (impairment of the hyperplastic-hypertrophic thyroid response to goitrogen treatment) (6, 3, 7, 8, 9). These effects have been interpreted as sequelae of depressed gonadotrophin and thyrotrophin secretion (3, 5, 7, 8). Although such lesions may appear virtually to abolish gonadotrophin secretion, producing a degree of testicular atrophy approaching that found after hypophysectomy, they have not (in the rat) been found to eliminate thyrotrophin secretion completely (8, 9). Evidence that they may depress corticotrophin secretion severely enough to permit marked atrophy of the adrenal cortex has not been abundant, but this effect has been noted also (8, 10). Further observations on lesion-induced adrenal atrophy are included in the present report.

These effects of hypothalamic lesions, although frequently multiple, appear to be selective (3, 7, 8, 11). A basic question is whether the lesions disrupt, severally or collectively, specific neural mechanisms concerned with the secretion of individual pituitary hormones. The study reported here was designed to answer this question.

If there are no such individual (presumably regulatory) hypothalamic-hypophyseal trophic mechanisms, then alternative explanations must be offered for the apparently selective lesion effects. It might be hypothesized that the lesions impair a single pan-adenohypophyseal sustaining mechanism, but that the various pituitary-target organ axes differ in their sensitivity to privation of this factor. From studies on the effects of fractional hypophysectomy in the dog (12) and rat (13), the gonad appears to be more sensitive to partial panhypopituitarism than either the thyroid or adrenal gland. In view of this, the selective production of "goiter block," or adrenal atrophy (i.e., impairment of thyrotrophin or corticotrophin secretion), by lesions which spare gonadotrophin secretion, argues against the concept that a pan-pituitary trophic mechanism is impinged upon. How-

Received May 8, 1956.

[1] Supported by a grant (B-708) from the National Institute of Neurological Diseases and Blindness, National Institutes of Health.

ever, since testicular maintenance, per se, is not adequate evidence of unimpaired gonadotrophin secretion (8), there remains a possibility that, in the lesion-bearing rats previously found to exhibit "goiter block" (3, 7, 8) or adrenal atrophy (11), but not gonad atrophy, the secretion of gonadotrophin also might have been greatly (perhaps equally) curtailed. Because of this possibility, the gonadotrophic function of such rats has now been examined by means of pituitary bioassays, which have been found to be a more sensitive index of gonadotrophin secretion than measurements of testicular maintenance alone (8).

MATERIALS AND METHODS

Hypothalamic lesions were made in 3–4 month old male Holtzman rats, 235–400 gm. in weight. Several series of about 20 rats each, closely grouped as to age and weight, were prepared with the aid of a Krieg-Johnson stereotaxic apparatus, nichrome wire electrode, and D-C power supply,[2] using a current of 2.5–3.5 milliamperes for 20–30 seconds to produce each lesion. The lesions, bilaterally paired, were placed close to the midline, at the diencephalic floor, at 0.3 to 0.5 mm. intervals between the optic chiasm and mammillary body.

In each series, 2–5 unoperated and/or sham operated controls, and 12–20 rats with lesions in a variety of sites, were prepared within 3 days. Thereafter they were housed in individual cages and maintained on Purina fox chow and tap water, *ad libitum*. After a 6–10 day "recovery" period, 0.15% propylthiouracil (PTU)[3] was given in the fox chow, and the rats were weighed at weekly intervals to evaluate food (and drug) consumption. After 3 weeks on this goitrogenic regimen, the survivors were killed with ether, and testes, seminal vesicles, ventral prostate, thyroid, and adrenal glands weighed and processed for histologic examination. Hypophyses were divided in the mid-sagittal plane; the left half was weighed and stored in the frozen state for subsequent bioassay, and the right half was fixed (without weighing) and processed for histologic study. Brains were removed whole and placed in 10% neutral formalin for several hours. The diencephalic region was then trimmed, fixed further (at least 4 days), dehydrated and embedded in tissuemat. Coronal sections, 10 μ. thick, were mounted serially at intervals of 100 μ. and stained with thionine for lesion localization studies.

Gonadotrophin bioassays were carried out as reported previously (8). Immature female Holtzman rats, born in this laboratory, served as recipients. Each half-pituitary, homogenized in saline, was given in six equal doses, every 12 hours, to a single recipient. The recipients were sacrificed 12 hours after the sixth injection, and their ovaries and uteri (emptied of fluid) weighed. Littermates served as uninjected controls and as recipients for control donors.

The method of Student's t was used in calculating P values.

RESULTS

Organ Weights. The half-pituitary, target organ, and body weight data, with statistical treatment, are shown in Table 1. Seven rats (Group I) did not appear to have suffered any ill effects as far as target organ weights were concerned, although there may have been some reduction in ventral

[2] The power supply unit was designed and constructed by Mr. Charles Schaffer.

[3] Dr. R. J. Turner of the American Cyanamid Company generously donated the propylthiouracil.

prostate size ($p = .025$). The 10 rats in Group II exhibited both "goiter block" (thyroid weight less than 30 mg.) and testicular atrophy, and cannot be considered as evidence for any selectivity of lesion effects. Two of these rats also showed adrenal atrophy.

Ten rats in Group III had goiters as large as the controls. Their testes showed atrophy which, although less severe than was found in Group II, was statistically significant. However, in view of the reported sequence of target organ manifestations of pan-pituitary privation (12, 13), these data might be considered compatible with an interpretation of partial panhypopituitarism, due either to incomplete removal of a non-specific

TABLE 1. MEAN BODY, TARGET ORGAN, AND HALF-PITUITARY WEIGHTS OF PTU-FED, LESION-BEARING AND CONTROL, MALE RATS

Group	No. of rats	Body wt. (gm.)	Organ weights (mg.) Testis	Sem. ves.	Vent. pros.	Thyroid	Adrenal	½ Pit.
Cont.	17	326 (280–365)†	3369 ± 208.5‡ (3065–3775)	321 ± 12.4 (245–405)	449 ± 22.9 (300–650)	49.6 ± 2.83 (39–77)	34.9 ± 0.87 (29–42)	5.6 (5.0–7.0)
I	7	319 (255–380)	3294 (2860–3700)	322 (250–360)	365 ± 27.6** (285–470)	49.0 (41–65)	39.9 (33–48)	5.3 (4.5–6.5)
II	10	305 (232–430)	948 (630–1800)	104 (65–250)	78 (29–206)	18.0 (9–28)	26.3 (17–38)	3.6 (1.5–5.2)
III	10	306 (260–345)	2107 ± 203.3* (1000–2635)	169 ± 12.9* (100–237)	174 ± 14.0* (100–260)	45.4 ± 1.87*** (40–61)	38.1 (28–46)	4.4 (2.0–6.0)
IV	10	275 (200–380)	3115 ± 89.3*** (2700–3500)	355 ± 25.9*** (240–460)	517 ± 51.3*** (278–765)	21.8 ± 1.67* (15–29)	27.8 (15–40)	4.1 (3.0–6.0)

† Range.
‡ Standard error.
Significance of difference from controls: *P < .001
**P < .1 > .001
***P > .1

adenohypophyseal trophic mechanism, or else to gross damage to the pituitary gland. The latter possibility seems unlikely in view of the half-pituitary weights in this group, and the fact that large infarcts were not seen in any of the histologically examined half-pituitaries.

In contrast to the rats in Group III, the ten rats in Group IV all had "goiter block." In some instances this was less extensive than that which appeared in Group II, but it was unequivocal in every case. None of these rats appeared to exhibit gonadal atrophy. In fact, the accessory sex glands of some were actually larger than those of any of the controls. It is noteworthy that the adrenal cortices of two rats in this group were atrophic (Table 2).

Gonadotrophin Assays. In Table 2 are the autopsy data for eight control donors (pooled) and for the 10 individual donors of Group IV. Also shown are the ovarian and uterine weights of their recipients, and of 11 uninjected recipients. This table includes the donor weights at sacrifice and their weight changes during PTU feeding, which are presented to demonstrate that the smaller thyroids in this group are not attributable to inadequate dietary goitrogen intake.

Administration of the half-pituitaries from 5 of the rats in Group IV produced no recipient ovarian response. In fact, 3 of these half-glands failed to elicit even a uterine response. These findings are in agreement with the previous study on the pituitary gonadotrophin content of lesion-bearing rats (8).

However, 5 of the donors appeared to have pituitary gonadotrophin stores which were within the control range. Their identifying symbols and recipient ovarian weights are italicized in Table 2. These rats comprise Sub-group IVa. The remainder make up Group IVb. Although the mean

TABLE 2. BODY AND TARGET ORGAN WEIGHTS AND PITUITARY GONADOTROPHIN ASSAYS FOR POOLED CONTROLS AND INDIVIDUAL RATS OF GROUP IV

	Donors								Recipients	
	Body weight (gm.)		Organ weights (mg.)						Weights (mg.)	
	At sac.	Δ†	Testes	Sem. ves.	Vent. pros.	Thyroid	Adrenal	½ Pit.	Ovaries	Uteri
11 uninjected recipients	—	—	—	—	—	—	—	—	18.1 ±1.05* (12–23)§	53.4 ±3.38 (41–79)
8 unoperated controls	304 ±10.3	−17.3 (+15 to −40‡)	3345 ±89.5	308 ±16.5	443 ±9.2	47.1 ±2.29	33.8 ±4.42	5.6 (5.0–7.0)	54.1 ±7.75 (26–90)	168.8 ±7.94 (133–201)
Group IV										
G8	232	+12	2700	269	278	28	31	3.0	*36*	176
G41	285	+15	3300	400	525	26	30	4.9	21	138
J3	340	+25	2900	460	765	20	18	4.0	*33*	142
J4	310	−20	3200	325	675	19	26	6.0	*31*	200
J6	250	−50	3500	430	425	29	26	3.5	23	166
J7	200	−75	3050	240	300	15	23	3.5	16	60
J9	260	−25	2900	275	450	16	15	3.2	19	60
J12	275	−35	3350	375	650	24	33	5.0	*36*	207
J15	220	−50	3450	315	450	25	36	4.0	*43*	195
K7	380	−40	2800	460	650	16	40	3.8	16	55
Group IVa	275	−13.6	3120 ±139.5 P=0.25	349	564 ±89.0 P=.017	23.2 ±1.66 P<.001	28.8	4.4	35.8 ±2.00 P=.11	184.0 ±11.7 P=.88

* Standard error.
† Weight change during PTU feeding.
‡ Other controls (unassayed) lost up to 75 gm.
§ Range.

testis weight for Group IVa is slightly lower than that for the controls, the difference was not statistically significant (P = 0.25), and would actually be reversed on a relative weight basis. On the other hand, the accessory sex gland weights were elevated. The .017 P value for the 27% (121 mg.) increase in mean ventral prostate weight suggests that the increase may be a real one. The 23.2 mg. mean thyroid weight for this sub-group is still significantly below the control value (P < .001).

Although the ovarian weight reponses for these 5 rats were not as great as the mean for controls, they were within the control range. The divergence of testis and accessory sex gland weights suggests a shift in FSH/LH ratios. In view of this, and the limited nature of these assays, it is not possible to estimate very precisely the degree to which gonadotrophin stores may have been lower in these rats than in the controls. However, in preliminary dose response studies on similar recipients, the weight response

to one-third of a control donor hypophysis was of the same order as that elicited by the half-hypophyses of the donors in Group IVa. Although FSH secretion may have been depressed to some extent, there is no evidence that LH production was, and the total gonadotrophin secretion in these 5 rats did not appear to differ remarkably from control values.

Histologic Observations. The adenohypophyses have not been studied microscopically in every animal, but they were examined in all of the rats of Group IV, and in these, as in the previous study (8), the PAS-staining gonadotrophs appeared as abundant and well stained as those of their controls. However, there seemed to be considerable difference in the intensity with which these cells could be stained in the controls of the various series of rats. Within a given series, on the other hand, there was little variation among the controls. Whether the wider differences between groups might be due to seasonal variations, or uncontrolled variations between different batches of diets, remains to be determined. No comparable variation was observed in the assayable pituitary gonadotrophic potency of controls from the various series.

Thyroidectomy cells were present in, and acidophile granulation absent from, all of the anterior lobes studied. Except in a few cases, the thyroid glands were not examined microscopically, since they were all hyperemic to gross inspection and would doubtless have appeared stimulated under the microscope. The testes of all group IV rats appeared normal.

Adrenal glands weighing less than 20 mg. were inspected microscopically. These atrophic glands showed similar changes (atrophy of the reticular and fascicular zones of the cortex) to those previously described and depicted in lesion-bearing rats with panhypopituitarism (8).

The histology of the posterior and intermediate pituitary lobes could not be assessed reliably because this region of the hypophysis was usually somewhat damaged in dividing the gland.

Lesion Localization. The lesions are best presented pictorially. A diagram of the rat hypothalamus, indicating in two planes the position and size of major landmarks, is shown in Figure 1. Outlines of the lesions of Groups II–IV have been superimposed on this diagram (Fig. 2) These sketches illustrate: (a) the regions completely *spared* by the particular group of lesions; (b) the zone *common to all* the lesions of that group; and (c) the configurations of typical lesions within the group, few of which individually were as large as the volume affected by the group as a whole. There was considerable variation within each group of lesions. However, all of the effective ones were basal, and many of them adhered to the floor of the skull, leaving an excavation in the brain (bounded by a rim of injured tissue) to mark their position and size.

The largest lesions were those of Group II (Fig. 2, I), which resulted in both "goiter block" and gonad atrophy. With a single exception, they destroyed virtually all of the anterior, and most of the posterior, hypo-

Administration of the half-pituitaries from 5 of the rats in Group IV produced no recipient ovarian response. In fact, 3 of these half-glands failed to elicit even a uterine response. These findings are in agreement with the previous study on the pituitary gonadotrophin content of lesion-bearing rats (8).

However, 5 of the donors appeared to have pituitary gonadotrophin stores which were within the control range. Their identifying symbols and recipient ovarian weights are italicized in Table 2. These rats comprise Sub-group IVa. The remainder make up Group IVb. Although the mean

TABLE 2. BODY AND TARGET ORGAN WEIGHTS AND PITUITARY GONADOTROPHIN ASSAYS FOR POOLED CONTROLS AND INDIVIDUAL RATS OF GROUP IV

	Donors								Recipients	
	Body weight (gm.)		♂ Organ weights (mg.)						Weights (mg.)	
	At sac.	Δ†	Testes	Sem. ves.	Vent. pros.	Thyroid	Adrenal	¼ Pit.	Ovaries	Uteri
11 uninjected recipients	—	—	—	—	—	—	—	—	18.1 ±1.05* (12–23)§	53.4 ±3.38 (41–79)
8 unoperated controls	304 ±10.3	−17.3 (+15 to −40‡)	3345 ±89.5	308 ±16.5	443 ±9.2	47.1 ±2.29	33.8 ±4.42	5.6 (5.0–7.0)	54.1 ±7.75 (26–90)	168.8 ±7.94 (133–201)
Group IV										
G8	232	+12	2700	269	278	28	31	3.0	*36*	176
G41	285	+15	3300	400	525	26	30	4.9	21	138
J3	340	+25	2900	460	765	20	18	4.0	*33*	142
J4	310	−20	3200	325	675	19	26	6.0	*31*	200
J6	250	−50	3500	430	425	29	26	3.5	23	166
J7	200	−75	3050	240	300	15	23	3.5	16	60
J9	260	−25	2900	275	450	16	15	3.2	19	60
J12	275	−35	3350	375	650	24	33	5.0	*36*	207
J15	220	−50	3450	315	450	25	36	4.0	*43*	195
K7	380	−40	2800	460	650	16	40	3.8	16	55
Group IVa	275	−13.6	3120 ±139.5 P=0.25	349	564 ±89.0 P=.017	23.2 ±1.66 P<.001	28.8	4.4	35.8 ±2.00 P=.11	184.0 ±11.7 P=.88

* Standard error.
† Weight change during PTU feeding.
‡ Other controls (unassayed) lost up to 75 gm.
§ Range.

testis weight for Group IVa is slightly lower than that for the controls, the difference was not statistically significant (P=0.25), and would actually be reversed on a relative weight basis. On the other hand, the accessory sex gland weights were elevated. The .017 P value for the 27% (121 mg.) increase in mean ventral prostate weight suggests that the increase may be a real one. The 23.2 mg. mean thyroid weight for this sub-group is still significantly below the control value (P<.001).

Although the ovarian weight reponses for these 5 rats were not as great as the mean for controls, they were within the control range. The divergence of testis and accessory sex gland weights suggests a shift in FSH/LH ratios. In view of this, and the limited nature of these assays, it is not possible to estimate very precisely the degree to which gonadotrophin stores may have been lower in these rats than in the controls. However, in preliminary dose response studies on similar recipients, the weight response

to one-third of a control donor hypophysis was of the same order as that elicited by the half-hypophyses of the donors in Group IVa. Although FSH secretion may have been depressed to some extent, there is no evidence that LH production was, and the total gonadotrophin secretion in these 5 rats did not appear to differ remarkably from control values.

Histologic Observations. The adenohypophyses have not been studied microscopically in every animal, but they were examined in all of the rats of Group IV, and in these, as in the previous study (8), the PAS-staining gonadotrophs appeared as abundant and well stained as those of their controls. However, there seemed to be considerable difference in the intensity with which these cells could be stained in the controls of the various series of rats. Within a given series, on the other hand, there was little variation among the controls. Whether the wider differences between groups might be due to seasonal variations, or uncontrolled variations between different batches of diets, remains to be determined. No comparable variation was observed in the assayable pituitary gonadotrophic potency of controls from the various series.

Thyroidectomy cells were present in, and acidophile granulation absent from, all of the anterior lobes studied. Except in a few cases, the thyroid glands were not examined microscopically, since they were all hyperemic to gross inspection and would doubtless have appeared stimulated under the microscope. The testes of all group IV rats appeared normal.

Adrenal glands weighing less than 20 mg. were inspected microscopically. These atrophic glands showed similar changes (atrophy of the reticular and fascicular zones of the cortex) to those previously described and depicted in lesion-bearing rats with panhypopituitarism (8).

The histology of the posterior and intermediate pituitary lobes could not be assessed reliably because this region of the hypophysis was usually somewhat damaged in dividing the gland.

Lesion Localization. The lesions are best presented pictorially. A diagram of the rat hypothalamus, indicating in two planes the position and size of major landmarks, is shown in Figure 1. Outlines of the lesions of Groups II–IV have been superimposed on this diagram (Fig. 2) These sketches illustrate: (a) the regions completely *spared* by the particular group of lesions; (b) the zone *common to all* the lesions of that group; and (c) the configurations of typical lesions within the group, few of which individually were as large as the volume affected by the group as a whole. There was considerable variation within each group of lesions. However, all of the effective ones were basal, and many of them adhered to the floor of the skull, leaving an excavation in the brain (bounded by a rim of injured tissue) to mark their position and size.

The largest lesions were those of Group II (Fig. 2, I), which resulted in both "goiter block" and gonad atrophy. With a single exception, they destroyed virtually all of the anterior, and most of the posterior, hypo-

thalamus. A number of them extended dorsally into the thalamus. These lesions invariably fused in the midline over part, if not all, of their length. Were it not for the exception mentioned above, the region common to all of the lesions of this group would be considerably larger.

The lesions in Group III (Fig. 2, II) were either smaller than those of

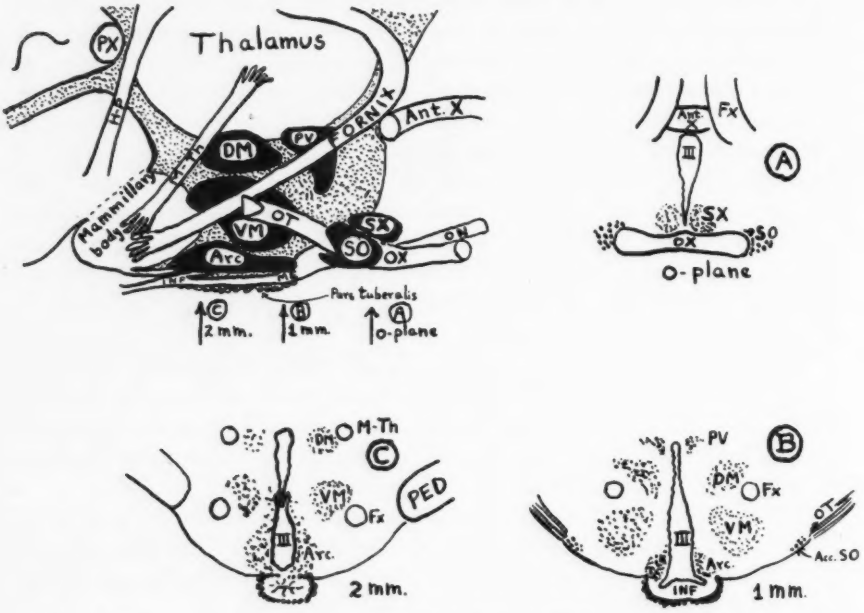


FIG. 1. Scheme of rat hypothalamus. Upper left: sagittal section through third ventricle (stippled), with nuclei and fiber tracts lateral to the midline superimposed by projection. A, B, and C: transverse sections at levels indicated by arrows below sagittal section. *Symbols*: ON, OX, OT—optic nerve, chiasm and tract. Nuclei: SX—suprachiasmatic, SO—supraoptic, Acc. SO—accessory supraoptic, PV—paraventricular, DM—dorsomedian, VM—ventromedian, and Arc.—arcuate, respectively. FX—fornix; M-Th and H-P—mammilothalamic and habenulopeduncular tracts; AX and PX—anterior and posterior commissures.

Group II, or else centered more caudally, so that they spared the anterior hypothalamus. Like those of Group II, however, they were fused in the midline throughout much of their length, destroying a large part, or all, of the basal periventricular region (in which the arcuate nuclei lie). In many cases there was localized, sometimes unilateral, evagination of the ventricular wall. Since this was not uniform, it indicates weakening of the wall itself, rather than increased cerebrospinal fluid pressure. These lesions also included many structures lateral to the periventricular zone, but no lesion has been found, in this or previous studies, to produce testicular atrophy *unless* it included a large part of the arcuate nucleus region.

The lesions of Groups IVa and IVb (Fig. 2, III and IV) were generally

smaller than the foregoing, and more anterior. They were centered $\frac{1}{2}$–$\frac{3}{4}$ mm. behind the optic chiasm. Although some of them extended caudally, they either bridged the arcuate nucleus region completely, or damaged it less (often unilaterally) than the lesions of Groups II and III.

The 4 lesions which produced adrenal atrophy were among the largest, damaging the anterior hypothalamus extensively and extending into the

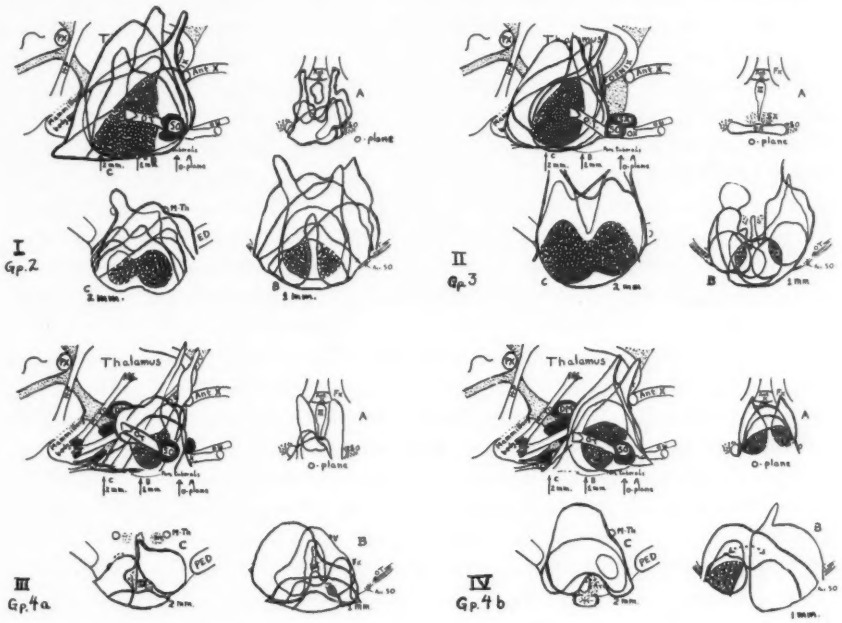


FIG. 2. Lesions for Groups II (UL), III (UR), IVa (LL), and IVb (LR), superimposed on diagram shown in Figure 1. Individual lesions are outlined in black. Areas common to all lesions in group are black with white dots.

thalamus. However, a number of seemingly equally extensive lesions have failed to produce this effect.

The lesions of group I have not been presented. They were either smaller than the majority described above, or as large but located primarily in the mammillary body. In either case, they wrought but slight damage to the arcuate nucleus region and spared the median eminence and anterior hypothalamus completely.

DISCUSSION

It would be difficult to explain the findings in this study on any basis other than selective damage, by the various groups of lesions, to specific neural mechanisms which support gonadotrophin and thyrotrophin secretion independently. However, the precise extent of such damage cannot always be evaluated by target organ studies alone. Thus, in half of the rats

of Group IV, which exhibited "goiter block" with unimpaired gonadal maintenance, pituitary bioassays revealed gonadotrophin secretion to be greatly impaired. Similar unrevealed damage to TSH and ACTH secretory mechanisms may well have existed in the rats of Group III, and the use of more stringent criteria of trophic hormone secretion will be necessary before identification of the specific neural mechanisms becomes possible.

This need for more direct criteria of anterior lobe function has been recognized by D'Angelo and Traum (9), who have estimated both stored and circulating TSH, as well as thyroid hormone secretion rates, in rats with anterior hypothalamic lesions. They found serum TSH, thyroid size and secretory rate all to be decreased by the lesions, whereas pituitary TSH stores were not. However, the lesions did not prevent the massive discharge of TSH in response to chronic goitrogen treatment. Their study fails to answer the question of whether the lesions influence TSH elaboration or release, or both, but it does suggest that lesion-impaired TSH secretion in the rat, in contrast to gonadotrophin secretion, is more readily reflected by circulating hormone levels and target organ response than by hypophyseal stores.

The question of whether lesions affect ACTH production or release must await further investigation. However, the marked adrenal cortical atrophy found in the present study suggests that at least one of these ACTH secretory processes is completely dependent (as gonadotrophin secretion appears to be) on the neural structures which control it.

The neural structures concerned with ACTH secretion must be more closely associated with those involved in TSH secretion than with the gonadotrophic mechanism(s). Neither here, nor in the previous study of Greer and Erwin (14), has adrenal atrophy been produced without "goiter block." Therefore, the concept that there are such individual TSH and ACTH secretion mechanisms is still only analogical, based upon the evidence that such separate mechanisms must exist for TSH and gonadotrophin secretion.

The failure of hypothalamic lesions to produce simultaneously complete and discrete effects on the adenohypophyseal secretory processes under neural control suggests interlocking or overlapping of the structures concerned. These structures cannot be defined precisely until the entire gamut of damage to them, from slight to total, can be measured reliably. However, almost total abolition of gonadotrophin secretion may be produced by lesions which appear to spare (largely, if not completely) the secretion of TSH and ACTH. These lesions do not involve the region of the hypothalamus anterior to the median eminence; neither do they involve the mammillary body. Conversely, the zone between the optic chiasm and the median eminence contains much, if not all, of the TSH and ACTH secretory-controlling mechanisms, and little, if any, of the gonadotrophin-supporting one(s).

The only hypothalamic nucleus which correlates positively with any anterior pituitary function is the arcuate nucleus. Although considerable damage to it is compatible with good testicular maintenance, its partial destruction has been found, nevertheless, to be accompanied by decreased gonadotrophic function (revealed by assays) despite the target organ maintenance. However, more evidence will be necessary to implicate the role of this nucleus per se in gonadotrophin secretion.

SUMMARY

Gonadotrophin assays, which appear to be the most sensitive index of gonadotrophin secretion, were carried out on the half-pituitaries of 10 lesion-bearing, goitrogen-treated rats which exhibited "goiter block," but not testicular or accessory sex gland atrophy. Adrenal cortical atrophy also was found in 2 of these rats.

This group of animals was selected for the assay studies because their target organ data suggested that thyrotrophin secretion had been impaired selectively, gonadotrophin secretion appearing to have been spared. This combination of effects appears to be incompatible with an interpretation of partial panhypopituitarism, and argues for the existence of separate hypothalamic-hypophyseal trophic (regulatory?) mechanisms concerned with the individual aspects of anterior lobe activity.

Although 5 of the 10 rats with apparently normal testicular maintenance proved, nevertheless, to have suffered extensive impairment of gonadotrophin secretion, as judged by the bioassays, this facet of anterior lobe function appeared to be only slightly, if at all, disturbed in the other 5. Furthermore, 1 of these 5 had adrenal cortical atrophy. The significance of these findings is discussed.

Acknowledgments

I wish to express my appreciation to Drs. J. M. Wolfe, S. A. D'Angelo, and N. S. Halmi for suggestions regarding the manuscript.

REFERENCES

1. Harris, G. W.: *Physiol Rev.* **28:** 139. 1948.
2. McCann, S. M.: *Am. J. Physiol.* **175:** 13. 1953.
3. Bogdanove, E. M. and N. S. Halmi: *Endocrinology* **53:** 274. 1953.
4. Hillarp, N. A.: *Acta Endocrinol.* **2:** 11. 1949.
5. Greer, M. A.: *Endocrinology* **53:** 380. 1953.
6. Greer, M. A.: *Proc. Soc. Exper. Biol. and Med.* **77:** 603. 1951.
7. Greer, M. A.: *J. Clin. Endocrinol. and Metab.* **12:** 1259. 1952.
8. Bogdanove, E. M., N. B. Spirtos and N. S. Halmi: *Endocrinology* **57:** 302. 1955.
9. D'Angelo, S. A. and R. E. Traum: *Endocrinology* **59:** 593. 1956.
10. McCann, A. M. and J. R. Brobeck: *Proc. Soc. Exper. Biol. and Med.* **87:** 318. 1954.
11. McCann, S. M.: Personal Communication.
12. Ganong, W. F. and D. M. Hume: *Endocrinology* **59:** 293. 1956.
13. Smith, P. E.: *Anat. Rec.* **53:** 191. 1932.
14. Greer, M. A. and H. L. Erwin: *Endocrinology* **58:** 665. 1956.

TEMPORARY GROWTH HORMONE DIABETES IN THE TUBE-FED RAT[1]

JOHN A. OWEN, JR.[2] AND FRANK L. ENGEL[3]

Departments of Medicine and Physiology
Duke University, Durham, North Carolina

PREVIOUS studies from this laboratory concerning the diabetogenic action of growth hormone in the rat (1, 2) were influenced by a concept first proposed by Young (3) that growth and diabetes are alternate responses to growth hormone. In these terms, diabetes does not develop in the rat receiving growth hormone because this species always has open epiphyses and hence is capable of responding with growth. Indirect support for this concept came in our observation that the force-fed rat developed temporary diabetes from growth hormone treatment if its growth response was compromised by the simultaneous administration of a subdiabetogenic dose of either ACTH or cortisone.

Young, however, has also suggested another explanation for the insensitivity of the rat as compared to the dog and cat. This relates to the remarkable ability of the rat's pancreas to maintain the integrity of its islet cells under the strain of a need for a sustained high rate of secretion. In the dog and cat this ability apparently is limited. There is good evidence that islet cell failure is the basis for the diabetes resulting from growth hormone treatment in these species.

During the course of further investigation of the diabetogenic action of growth hormone in the rat, it was found that occasionally tube-fed rats developed glycosuria with growth hormone treatment under circumstances in which there was no reason to assume that growth inhibition would occur prior to the development of the diabetes. The present report describes these experiments and offers an explanation within the framework of Young's two concepts.

METHODS AND MATERIALS

Male albino rats of the Vanderbilt strain were housed in metabolism cages in an air-conditioned room at 27° C. They received tap water to drink *ad libitum* and were tube

Received August 1, 1956.

[1] Presented at the 38th Meeting of the Endocrine Society, June 9, 1956, Chicago, Illinois.

[2] American Diabetes Association fellow. Present address: Department of Internal Medicine, Medical College of Georgia, Augusta, Georgia.

[3] Supported by research grants from the Duke University Research Council and the American Cancer Society, administered by the Committee on Growth, The National Research Council.

fed twice daily a high carbonydrate liquid diet containing 0.45 gm. carbohydrate, 0.07 gm. protein, 0.009 gm. fat and 2.16 calories per ml. in addition to minerals, vitamins and an antibiotic as recorded in a previous report (2). The rats were adapted to the tube feeding in 4 to 7 days by gradually increasing the daily volumes of diet until the full volume of 5.5 ml./100 gm. body weight per feeding was reached. The rats were not used for the experiments until at least 10 days of full tube feeding had elapsed. All animals were weighed daily and, except where indicated, the volume of diet was given on the basis of that day's weight.

The principal growth hormone preparation was Horner's lot PR-1 prepared from pork piutitaries by the technique of Raben and Westermeyer (4). It was prepared for injection by dissolving in glass distilled water acidified to pH 3.5–4.0 by the dropwise addition of 0.2 N HCl. The final concentration was 10 mg. of powder per ml. An Armour beef growth hormone (Lot R-491024) was dissolved in distilled water, brought to pH 9.5 with 0.1N NaOH. As a control, commercial human serum albumen solution was diluted and adjusted to a pH and concentration identical with those for the PR-1.

Hormone solutions were prepared freshly for each course of treatment and administered for 5 consecutive days by subcutaneous injection immediately after the morning feeding. Blood was obtained from the tail without anesthesia 3 hours after the morning feeding and analyzed for sugar by the method of Somogyi (5). Twenty-four hour collections of urine preserved with thymol were analyzed for reducing substances according to Benedict (6).

RESULTS

In the first experiment, illustrated in Figure 1, 12 rats (Series A) with a mean body weight of 413 grams were tube fed an average of 44.5 ml. of diet daily (11 ml./100 gm.) for 9 days, during the last 5 of which they received 2 mg. of growth hormone per 100 gm. body weight, or an average daily dose of 8.3 mg. per rat. For comparison 7 rats (Series B) with a mean body weight of 459 gm. were fed the same diet load (11 ml./100 gm.) but received daily injections of serum albumen (9.1 mg.) as an inert protein for control. Figure 1 shows that during the control period of tube feeding both groups of rats exhibited good rates of weight gain and had no significant glycosuria or hyperglycemia as estimated 3 hours after tube feeding. The albumen injections in Series B had no influence on any of the parameters studied. In contrast, the growth hormone injection induced a prompt and statistically highly significant increase in both glycemia and glycosuria. There was a weight spurt during the first 24 hours, but thereafter the slope of the weight curve was comparable to that before treatment.

This response was at variance with our previous claim that growth hormone did not induce diabetes in the tube fed rat unless the latter was simultaneously treated with sub-diabetogenic doses of cortisone or ACTH. In seeking an explanation for this discrepancy, we were struck by the fact that 3 of the 12 rats in Series A did not develop diabetes. These were the smallest, all weighing less than 390 grams. In this and most of the previous work both diet and growth hormone were administered per unit of body weight and hence the three non-diabetic animals received a smaller total

dose of hormone and volume of diet. The following experiments were designed to clarify the relative significance of the three variables, i.e. body weight, dose of hormone and caloric intake.

In the next experiment body weight was made the variable and the dose of hormone and the volume of diet were relatively constant (Fig. 2). Six rats with mean weight of 350 grams (Series C) received 38 ml. of diet (11

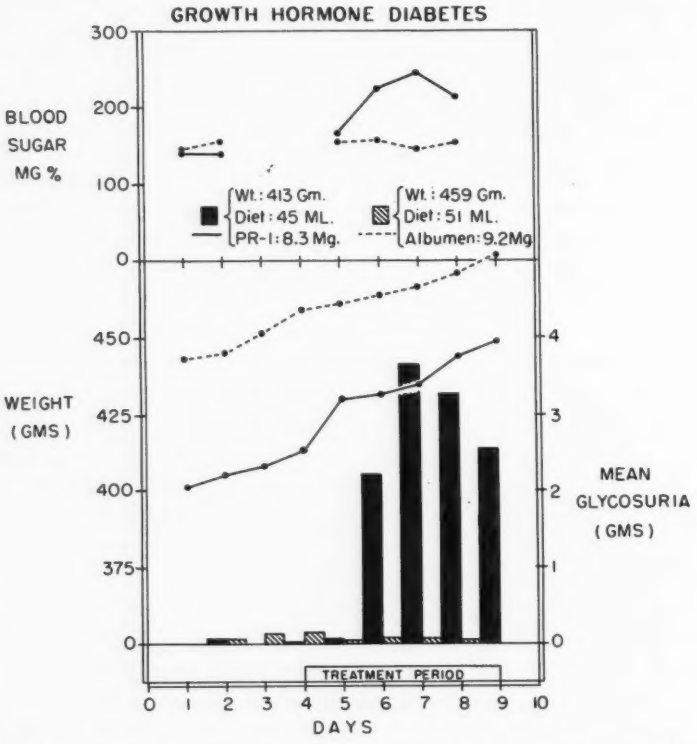


FIG. 1. Temporary diabetes in the rat receiving growth hormone (PR-1), Series A, compared to albumen control, Series B.

ml./100 gm.) and a mean daily dose of 8.8 mg. of growth hormone (2.5 mg./100 gm.) from the fourth to the ninth days. Although some glycosuria resulted, it was less than that seen in the previous experiment and the blood sugar levels measured 3 hours after tube feeding were not significantly different from the pretreatment or albumen controls (Series B, Fig. 1). In contrast to Series A, there was a sustained increase in the weight curve.

At the conclusion of the growth hormone period the rats' diet was restricted to a constant volume (38 ml.) for several weeks until their body

weights reached approximately those of the diabetic animals in Series A, i.e., 425 gm. The study was then repeated, using the identical diet volume and hormone dosage as had been used previously when the rats were smaller. The results were essentially the same demonstrating that the capacity to develop diabetes was not a function of the weight or, presumably the age

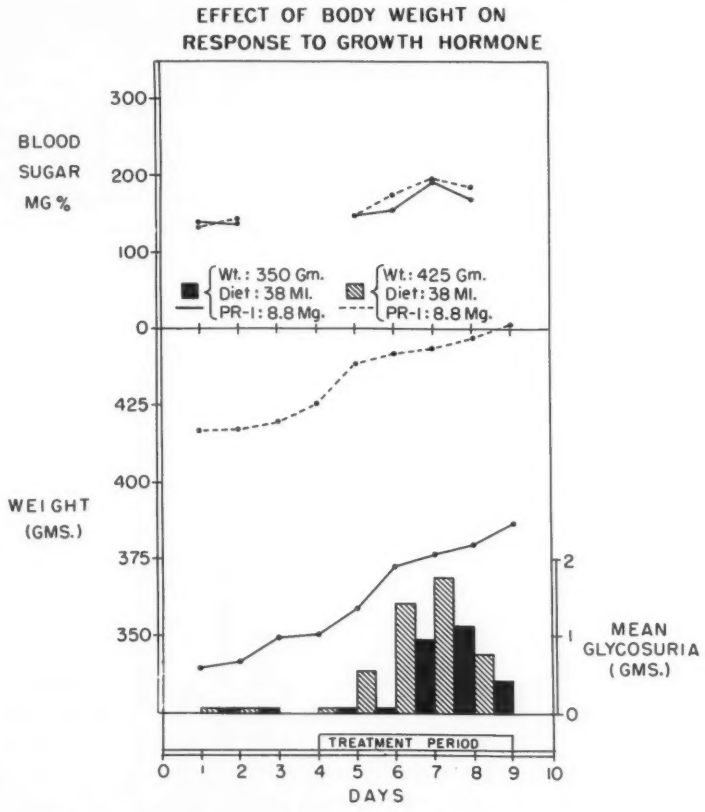


FIG. 2. Series C. With the dose of growth hormone and the total volume of diet kept constant, mild diabetes results in both experiments even though the body weight had increased by 75 grams.

of the rat (Fig. 2). Moreover, since these rats received a larger dose of growth hormone than did those in Series A, the dose of hormone, at least within this range was apparently not critical.

These results indicated that the total food intake was the determining factor, since in the last experiment Series C differed from Series A only in this respect. To test this a series of 4 small rats (D) was fed a gradually increasing volume of diet for several weeks until they were receiving 45 ml.

when they weighed 355 grams. With this diet they showed no spontaneous hyperglycemia or glycosuria (Fig. 3). When given 8.9 mg. of growth hormone, the same daily dose as administered to Series C, they developed marked hyperglycemia and glycosuria and their weight curves showed no change in slope during the period of hormone administration. The degree of glycemia and glycosuria was comparable to that in Series A, which is plotted in Figure 3 for comparison. The blood sugar levels in Series A and D were statistically significantly greater than in the albumen controls ($p < .01$) and in both experiments of Series C ($p < .02$).

The diabetic response noted with the Raben-Westermeyer growth hormone was also found when the Armour growth hormone was injected in doses of 1.5 and 2.0 mg. per 100 grams in a group of rats comparable to Series A. Indeed, the diabetes was even more severe, with a high mortality, suggesting that this preparation had greater diabetogenic potency than the Raben-Westermeyer material used in the bulk of these experiments.

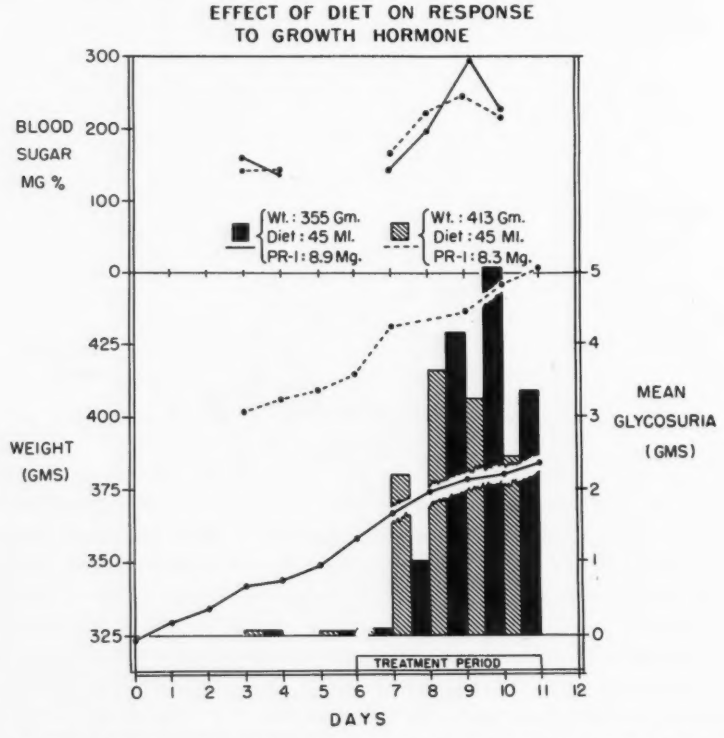


FIG. 3. The administration of excessive diet to smaller rats, Series D black column, results in an even greater degree of diabetes than occurred in the larger rats of Series A receiving the same diet.

DISCUSSION

These data demonstrate that a severe temporary hyperglycemic-glycosuric response can be induced in the rat given growth hormone if the food intake is excessive. The diabetes became apparent when the daily carbohydrate intake reached 20 grams. It will be recalled that Ingle (7) showed in tube feeding experiments that the tolerance to carbohydrate of the normal rat before glycosuria resulted was 26–29 grams of carbohydrate, including that derived from gluconeogenesis. Without growth hormone our rats tolerated 20 grams of dietary carbohydrate without difficulty and showed a steady rate of weight gain.

Nitrogen balances unfortunately were not estimated in these particular experiments and hence for them it is not possible to equate weight increments with growth. However, in another series of animals comparable to Series D, more prolonged treatment with growth hormone resulted in a simultaneous increase both in weight gain and in nitrogen balance. The ratio of total nitrogen retained to total weight gained during the entire 18-day treatment period averaged 16.4 mg. N/gram weight. Assuming a comparable balance in the present experiments, it would seem inescapable that diabetes occurred despite some growth response. Similar data are found in the report of Batts *et al.* (8).

If this interpretation is correct, then Young's second explanation would best fit the data, e.g. the excess food consumption plus growth hormone may have led to a situation in which the islet cells were temporarily incapable of supplying an amount of insulin sufficient to meet the needs for both growth and the utilization of the excess carbohydrate load. Since insulin is necessary for the optimal growth promoting potential of growth hormone to become manifest (9), it would be anticipated that as soon as insulin secretion became limiting the growth response would fail. So long as the intact rat remained resistant to the diabetogenic action of growth hormone, it was difficult to decide which was of primary significance, the capacity for further growth inherent in open epiphyses or the well known sturdiness and regenerative ability of the pancreatic islets in this species. The present observations plus unpublished studies which show that the diabetes is temporary despite continued overfeeding favor the concept that the secretion of insulin is the key factor. This conclusion is also supported by the recent report of Batts *et al.* (8) showing that growth hormone stimulates islet cell regeneration in the partially pancreatectomized rat. An initial accentuation of diabetes in these experiments was followed by an amelioration of the glycosuria.

Investigation currently in progress in this laboratory on nitrogen balance and on the influence of changing the proportions of protein, carbohydrate and fat in the diet on the diabetongenic action of growth hormone, should help resolve some of these questions.

SUMMARY

Rats tube-fed an excessive diet high in carbohydrate develop temporary diabetes when administered growth hormone.

The significance of this observation is discussed in light of current concepts of the mechanism of the diabetogenic action of growth hormone.

Acknowledgments

The authors wish to express their gratitude to Dr. Len Mitchell of the Horner Co., Ltd., of Montreal, and to Dr. Irby Bunding of the Armour Laboratories for generous supplies of growth hormone.

REFERENCES

1. ENGEL, F. L., A. VIAU, W. COGGINS AND W. S. LYNN, JR.: *Endocrinology* **50**: 100. 1952.
2. BOGDONOFF, M. D., E. H. SCHERR, L. LISTER, J. A. OWEN, JR. AND F. L. ENGEL: *Endocrinology* **57**: 272. 1955.
3. YOUNG, F. G.: *J. Clin. Endocrinol. and Metab.* **11**: 531. 1951.
4. RABEN, M. AND V. W. WESTERMEYER: *Proc. Soc. Exp. Biol. & Med.* **78**: 550. 1951.
5. SOMOGYI, M.: *J. Biol. Chem.* **160**: 61. 1945.
6. BENEDICT, S. R.: *J.A.M.A.* **57**: 1193. 1911.
7. INGLE, D. J.: *Endocrinology* **39**: 43. 1946.
8. BATTS, A. A., L. L. BENNETT, S. ELLIS AND R. A. GEORGE: *Endocrinology*, **59**: 620. 1956.
9. MILMAN, A. E., P. DEMOOR AND F. D. W. LUKENS: *Amer. J. Physiol.* **166**: 354. 1951.

CORTICOSTEROID METABOLISM IN LIVER: *IN VIVO* METABOLISM OF CORTISONE AND CORTISOL BY DOG LIVER[1]

O. HECHTER, E. FRANK, E. CASPI AND H. FRANK

Worcester Foundation for Experimental Biology, Shrewsbury, Mass., Kirstein Laboratory of Surgical Research, Beth Israel Hospital and Department of Surgery, Harvard Medical School, Boston, Massachusetts

CORTICOSTEROIDS like cortisone and cortisol, when administered by the oral route are absorbed from the gut via the portal vein (Bayliss, *et al.*, 1954; Kass *et al.*, 1955). It is therefore apparent that orally administered corticoids pass through the liver before entering the systemic circulation. The mammalian liver has a great capacity for transforming cortisone and cortisol to other products (for references *cf.* Dorfman and Ungar, 1953) and the question then arises as to what extent corticoids entering the liver via the portal vein escape the hepatic metabolic process and emerge as unaltered cortisone or cortisol. This question is of particular importance in relation to the well established fact that the biological activity of cortisone and cortisol is almost as great when given by the oral route as parenterally (Thorn *et al.*, 1951).

Nelson and Harding (1952) have previously reported in a short note that corticoids disappear following their infusion into a peripheral vein, as evidenced by a significant drop in the concentration of 17-hydroxycorticoids in hepatic venous blood relative to portal venous or arterial blood drawn simultaneously. Although there were variations, 17-hydroxycorticoid concentration measured by the Nelson-Samuels (1952) method in the hepatic vein was about 50% of that in the portal venous or arterial blood (D. H. Nelson, personal communication). Since the hepatic blood flow in these experiments was not reported, it is not possible to calculate steroid disappearance rates (A–V difference $\times$ blood flow) from these data.

To obtain an estimate of the order of magnitude of the hepatic catabolism of orally absorbed corticoids, cortisone-4-C^{14} has been administered into the portal vein of one dog and cortisol-4-C^{14} into another, and the rate of disappearance of added corticoid consequent to circulation through the liver has been studied. The results to be presented demonstrate that the major portion of the corticoid administered under our conditions is not recoverable from the hepatic venous outflow in unaltered form nor can it be found in the liver or bile.

Received August 31, 1956.

[1] This investigation was supported in part by U. S. Public Health Service Research Grants No. R.G.-2742 and H-1329.

METHOD

General: Cortisone-4-C^{14} and cortisol-4-C^{14} were infused continuously at a steady rate into the portal vein of two dogs. During the entire infusion the entire outflow from a lobar hepatic vein was collected and a continuous blood sample was taken from the portal vein and the femoral artery. The blood samples, the bile collected during the period of infusion, and an aliquot of the liver obtained at the end of the experiment were then extracted and any cortisone or cortisol present was then detected by paper chromatography. it is possible to calculate hepatic catabolism rates of corticoid from the values for hepatic blood flow and the difference in corticoid content of portal venous and hepatic venous blood on the assumptions that: (1) 75% of the hepatic blood flow is derived from the portal vein and 25% from the hepatic artery, since Hahn *et al* (1945) have reported that 20–30% of hepatic blood flow is supplied by the hepatic artery, and (2) the blood flow and metabolic activity in the cannulated lobe is essentially the same as that which obtains in the rest of the liver.

Corticoids: Cortisol-4-C^{14} and cortisone-4-C^{14} (obtained from the Endocrinology Study Section, Division of Research Grants of U. S. Public Health Service) were purified by paper chromatography, mixed with carrier steroid and recrystallized. All samples for radioactivity were plated directly in stainless steel planchettes (1″ diam., 5/16″ in depth), counted in a windowless flow gas counter at infinite thinness; no more than 1 mg. of material was plated in any instance. Triplicate samples were plated and counted and the counts reported are accurate to $\pm 10\%$. The diluted cortisone-4-C^{14} (12.2×10^3 cts. per min. per mg.) and cortisol (9.8×10^3 cts. per min. per mg.) samples revealed only a single zone of radioactivity following paper chromatography in the chloroform-formamide system, and it was observed that about 99% of the total radioactivity in both samples was eluted from the respective cortisone or cortisol zones, indicated by the following tests: (a) blue and red tetrazolium tests for ketolic side chain (b) ultraviolet scanning of papergram and dinitrophenylhydrazine tests for Δ^4-3 ketone function and (c) H_2SO_4 fluorescence (for references, cf. Caspi *et al.*, 1953; Caspi and Hechter, 1956).

For experimentation the steroids were dissolved in absolute ethanol (10 mg. per ml.) and diluted dropwise with stirring in whole blood to a total volume of 1000 ml.

Preparation of Dogs for Experimentation: The dogs used were healthy mongrels and were prepared under morphine and ether as follows: A wide bore plastic catheter was passed through the jugular vein and vena cava and wedged into a lobar hepatic vein where it was held by an encircling ligature placed through an abdominal incision. Outflow from this catheter was collected for measurement of flow rate and for samples of hepatic venous blood. Before hepatic venous samples were required, the emerging hepatic venous blood was returned to the circulation via the femoral vein. Another large plastic catheter was passed through a mesenteric tributary into the portal vein for blood samples and a slender catheter was placed just proximal to the portal vein, via a tributary of the splenic vein for the introduction of steroids into the portal inflow. The cystic duct was ligated and the common bile duct catheterized for collection of all hepatic bile. The femoral artery on one side was cannulated for the measurement of arterial pressure by a mercury mannometer; the opposite femoral artery was exposed to permit sampling of arterial blood. One femoral vein was cannulated for transfusion. Heparin (50 mg.) was given when the hepatic venous catheter was placed and smaller doses of heparin were given at regular intervals thereafter.

All wounds were closed and the animal allowed to recover fully from anesthesia before the infusion was initiated which represented the start of the experiment. Then, all of the hepatic venous outflow from the catheter was collected; simultaneously the catheters for the sampling of the portal venous and femoral arterial blood were partially opened and a

fraction of the blood circulating in these vessels was collected over the entire period of infusion. Thus the steroid content of the samples collected represent a mean value over the entire period of the infusion. The large blood samples were continuously replaced by transfusions of donor blood from dogs so that the arterial pressure of the experimental animals was maintained at 110–120 mm. Hg. At the termination of the experiment, the catheters were checked and found to be in proper place; the segment of liver drained by the cannulated hepatic vein was delineated by retrograde injection of a mass which does not traverse sinusoids (Wessler and Schlesinger, 1953). The marked segment of liver was excised and weighed (minus the weight of injected mass) and the remainder of the liver was weighed.

Estimation of Cortisone and Cortisol Content of Samples: The collected blood samples, the bile, and a known aliquot of homogenized liver were extracted exhaustively with isopropyl acetate, to remove unconjugated steroids using procedures previously described (Caspi and Hechter, 1956). The lipoid residue thus obtained was then subjected to silica gel chromatography. The non-polar contaminants which did not contain appreciable radioactivity, were eluted with hexane, benzene and benzene-ethyl acetate mixtures (19:1); the radioactive compounds were stripped from the column with ethyl acetate and methanol. Samples of *unlabeled* cortisone and cortisol (2 mg. each) were then added to the steroid concentrate, and the residues were fractionated by paper chromatography in the chloroform-formamide system. The cortisone and cortisol zones were detected, rechromatographed and eluted and counted. It was possible from the count to calculate the amount of C^{14}-cortisone or C^{14}-cortisol present in the various samples since the specific activity of the administered C^{14}-corticoid is known. Following the addition of 25 to 100 mg. of C^{14}-cortisone or cortisol to 1 liter blood, the procedures used in these studies lead to recovery of 75–80% free cortisone or cortisol respectively.

RESULTS

Cortisone: 98.2 mg. of cortisone-4-C^{14} were infused at a constant rate for 60 minutes into the portal vein of a 40 kg. dog whose liver weighed 1326 gm. at the end of the experiment. The cannulated lobe weighed 34 g. and its hepatic venous outflow during this period was 640 ml. (hepatic blood flow = 0.31 ml. per gm. per min.). The C^{14}-cortisone and C^{14}-cortisol contents of the various samples of blood, liver and bile are shown in Table 1. It will be seen that about 4% of the cortisone infused into the portal vein emerges from the liver as unaltered cortisone or formed cortisol. The missing counts could not be accounted for in the bile or in the liver, hence the difference between portal vein and hepatic vein samples represents steroid

TABLE 1. CORTISONE TRANSFORMATION IN LIVER

Sample	Vol.	Cortisol* μg./100 ml.	Cortisone* μg./100 ml.
Portal vein	610	16	184
Hepatic venous	640	5	3
Femoral arterial	660	14	3
Liver	124	16	7
Bile		0	0

* Estimated on the assumption that 1 mg. cortisone or cortisol would have 12.2×10^3 cts. per min.

catabolism. The catabolism rate of cortisone, calculated upon the basis of our assumptions in the liver of this dog was about 563 μg. per min or 33.8 mg. per hour.

Cortisol: 102 mg. of cortisol-4-C^{14} were infused for 83 minutes into the portal vein of a 27 kg. dog whose liver weighed 470 gm. The hepatic venous outflow from a 26 gm. liver segment during this period was 1550 ml. (Hepatic blood flow = 0.72 ml. per gm. per min.). The C^{14}-cortisol content of the various samples of blood, liver and bile are shown in Table 2. About 20% of the cortisol infused into the portal vein emerges as free cortisol in the hepatic vein. The missing steroid could not be detected in the liver or bile as unaltered corticoids. The calculated catabolsim rate for cortisol was 493 μg. per min, or 29.6 mg. per hr. in this dog.

TABLE 2. CORTISOL TRANSFORMATION IN LIVER

Sample	Vol.	Cortisol* μg./100 ml.	Cortisone* μg./100 ml.
Portal vein	290	243	3
Hepatic venous	1550	48	3
Femoral arterial	365	17	5
Liver	100	0	4
Bile		0	0

* Estimated on the assumption that 1 mg. cortisol or cortisone would have 9.8×10^3 cts. per min.

DISCUSSION

It is not possible from the data of these two experiments to asssess the precise range of rates of catabolism of corticoids which enter the liver directly from the portal vein nor to evaluate any possible differences in this regard between cortisone and cortisol. However, the results do clearly demonstrate that under our experimental conditions these corticoids administered into the portal vein are in largest part metabolized by the liver to other substances. The rate of corticosteroid disappearance by the entire liver evaluated from the hepatic blood flow data and the difference in steroid concentration between the portal vein and the hepatic vein is calculated to be about 563 μg. per min, in the cortisone experiment; in the cortisol experiment the rate was about 493 μg. per min. which is a comparable order of magnitude. It thus would appear that under conditions where corticoid is infused into the portal vein to produce levels of about 200 μg. per 100 ml. that the corticoid catabolism rate by liver, calculated on the basis of our assumptions, is of the order of 525 μg. per min, or 31.5 mg. per hr. This calculation, however, is based upon the assumption of a constant contribution of 25% of hepatic arterial blood to the total hepatic flow. It is apparent that to the extent that the hepatic blood flow is from the hepatic artery (which contains only minor amounts of corticoid) in greater or smaller amount than assumed, the calculated rates of corticoid transformation are correspondingly influenced.

In the cortisol experiment about 20% of the administered steroid emerged from the liver as cortisol or cortisone; in the cortisone experiment this value was less than 4%. These apparent differences may mainly reflect the different rates of hepatic blood flow in the two experiments. In the cortisol experiment the hepatic blood flow rate was 0.72 ml. per gm. liver per min.; in the cortisone experiment the rate was only 0.31 ml./gm./min. The rate of steroid metabolism must depend not only upon the number of substrate molecules presented to the hepatic enzymatic apparatus for transformation per unit time but upon the period of contact of each substrate molecule with enzyme as well. When flow is slow the contact period is relatively long and change in blood level is greater than with rapid flow.

Independent estimates of the hepatic catabolism process using cortisol have been obtained in two dogs by Drs. G. Sayers and E. Selkurt (unpublished work).[2] Corticoid was infused into a peripheral vein at a steady rate of 1.0 mg. per minute for 3.5 hrs. in order to attain a "steady state"; samples of femoral artery, portal-vein and hepatic vein blood were then drawn at hourly intervals, and effective hepatic blood flow was determined by the bromsulphthalein method. The blood samples were analyzed for cortisol by paper chromatography. Under their conditions the concentration of corticoid in the portal vein was only slightly less than in arterial blood, thus differences between portal venous and hepatic arterial contribution to total hepatic blood flow do not significantly influence their calculated transformation rates. While these workers found appreciable concentrations of the cortisol in the hepatic vein in the "steady state," the transformation rates of cortisol calculated were 477 and 768 μg. per min. in two dogs whose mean hepatic blood flow rates were 427 and 975 ml. per min. respectively. These values, obtained under entirely different conditions, are in reasonably good agreement with the present study and suggest that the *in vivo* corticoid catabolic rate in dog liver ranges between 0.5 to 0.8 mg. per min. or 30 to 48 mg. per hour.

The corticoid disappearance as a result of circulation through liver has been shown not to be due to accumulation or retention of the administered corticoid in either liver or bile. The nature of the metabolites formed *in vivo* were not directly investigated in this study; however, it was observed that only that minor amounts of radioactive material more polar or less polar than cortisone or cortisol were directly extractable by isopropyl acetate from samples of hepatic vein blood, the liver, and bile. This suggests that the major portion of the administered C^{14}-corticoid was rendered highly water-soluble, probably by conjugation.

There remains to be considered the significance of these observations in relation to the known activity of orally administered cortisone. The ques-

---

[2] We should like to thank Drs. G. Sayers and E. Selkurt, Western Reserve University for the opportunity of examining their data, prior to publication.

tion previously raised (Hechter, 1953; Hechter *et al.*, 1953) as to why cortisone given orally retains almost all of its activity even though a large fraction is converted to products other than cortisone or cortisol remains unanswered and merits serious investigation.

SUMMARY

The rate of hepatic catabolism of cortisone-4-C^{14} and cortisol-4-C^{14}, administered at a steady rate into the portal vein has been studied in two dogs. At a corticoid concentration of about 200 μg. per 100 ml. portal vein blood, the corticoid catabolism rates by the entire liver are about 525 μg. per min. or 31.5 mg. per hour. The significance of this finding has been briefly discussed in relation to the effectiveness of orally administered cortisone, known to be absorbed from the intestines via the portal vein.

REFERENCES

Bayliss, R. I. S., W. J. Dempster, B. J. Round and A. W. Steinbeck: *Clin. Sci.* **13**: 377. 1954.

Caspi, E., H. Levy and O. Hechter: *Arch. Biochem. & Biophys.* **45**: 169. 1953.

Caspi, E. and O. Hechter: *Arch. Biochem. & Biophys.* **61**: 299. 1956.

Dorfman, R. I. and F. Ungar: "Metabolism of Steroid Hormones," Burgess, Minneapolis, 1953.

Hahn, P. F., W. D. Donald and R. C. Grier, Jr.: *Am. J. Physiol.* **143**: 105. 1945.

Hechter, O.: *Ciba Foundation Colloquia on Endocrinology* **7**: 272. 1953.

Hechter, O., M. M. Solomon and E. Caspi: *Endocrinology* **53**: 202. 1953.

Kass, E. H., O. Hechter, E. Y. Caspi and R. R. Aycock: *J. Clin. Endocrinol. and Metab.* **14**: 167. 1955.

Nelson, D. H. and B. Harding: *Federation Proc.* **11**: 379. 1952.

Nelson, D. H. and L. T. Samuels: *J. Clin. Endocrinol. and Metab.* **12**: 519. 1952.

Thorn, G. W., A. E. Renold, L. Wilson, T. F. Frawley, D. Jenkins, J. Garcia-Reyes, and P. H. Forsham: *New England J. Med.* **245**: 549. 1951.

Wessler, S. and M. J. Schlesinger: *Circulation* **7**: 641. 1953.

THE THYROID-ADRENAL RELATIONSHIP: THE EFFECT OF CORTISONE AND OF THYROID HORMONE ON HEPATIC NECROSIS OF DIETARY ORIGIN IN THE RAT[1]

K. ATERMAN

Department of Anatomy, University of Birmingham Medical School, Birmingham, England

MOST recent studies of the thyroid-adrenal relationship deal mainly with the effects of the thyroid or adrenocortical hormones on either gland and/or on the adenohypophysis. Little attention has been paid to the peripheral interrelations of these hormones. It is with this aspect of the thyroid-adrenal relationship that this paper is concerned.

The experiments presented here were prompted by observations showing that at least some of the effects of the prolonged administration of cortisone can be significantly modified by the simultaneous induction of a hyperthyroid state (1, 2, 3, 4). The explanation which best fitted these findings was based on the assumption that the increased metabolic requirements of the hyperthyroid state lead to increased "utilisation" and more rapid destruction, inactivation or excretion of the administered adrenocortical steroid, thus preventing the accumulation of an excess of it in the body. Recently more direct evidence has been presented in favour of this view (5, 6, 7). To see if this hypothesis also applied to certain pathological phenomena which are affected in a characteristic manner by cortisone, the following experimental series was devised in which the survival time of rats fed a deficient diet known to produce liver necrosis was studied.

METHODS

Four separate experiments, comprising a total of 160 male rats of an inbred strain used in the Department of Anatomy, were undertaken. Each experiment consisted of 4 treatment groups, to each of which one of four littermates was allocated. Twenty-one days after birth, on the first day of the experiment, the litters were weaned; each littermate was placed into a separate cage with raised screen floor in a room of fairly constant temperature (70° F.), and the treatments shown in Tables 1–4 were started. Although the initial weight of the experimental animals in the whole series ranged from 29–45 gm., the variation within one litter group was rarely more than 3–5 gm. Throughout the experiment the "necrogenic diet"[2] of Lindan and Himsworth (8) was used as the basic

Received September 10, 1956.

[1] Presented in part at the VIth International Congress of Anatomy, Paris, 1955.

[2] Dried yeast (United Yeast Co., Ltd.) 18%
Corn starch 73%
Olive oil 5%
Cod liver oil 1%
Salt mixture (Glaxo) 3%
Added vitamins per 10 gm. of food: Thiamin hydrochloride 20 mg., Pyridoxine 20 mg., Riboflavine 25 mg., Calc. Pantothenate 100 mg.

diet, which was given to all animals. One littermate received no other treatment. A second animal was given the diet into which thyroid powder (Parke, Davis Co.)[3] had been mixed at a level of 0.2%. The third littermate was given injections of cortisone acetate ("Cortone" Merck) subcutaneously each day at a level of 1 mg. (the "standard dose"), 2 mg. or 5 mg. per 100 gm. body weight, depending on the experiment. The fourth animal was treated with cortisone and with thyroid powder. Each rat injected with cortisone was weighed daily and the dose adjusted according to the change in body weight. Food intake was measured daily. In Experiments I and IV food and water were allowed *ad lib.*, but in Experiments II and III restricted feeding was adhered to in such a manner that only the animal given the diet containing 0.2% thyroid powder, the "leading animal," was allowed to eat as much as it liked whereas its 3 littermates were only given as much of their respective diets as the leading animal had eaten on the previous day. After the death of the leading animal its average daily food intake was calculated and only that amount was then given daily to the survivors. The presence or absence of liver necrosis was recorded at the time of death.

After these 4 experiments had been terminated, a fifth experiment was undertaken to see whether the conclusions arrived at in the preceding experiments also applied to rats treated with ACTH. For this purpose groups of 3 littermates each, weaned on the 21st day of life, the first day of the experiment, were allowed to eat the basic diet *ad lib.* On the 20th day of the experiment treatment with a long-acting preparation of adrenocorticotrophin was started in 2 rats by injecting them subcutaneously every other day with 1 unit of "Cortrophin Z" (Organon). One of these animals received in addition thyroid hormone by substituting the diet supplemented with 0.2% of thyroid powder for the basic diet. The third animal served as control.

RESULTS

Experiment I. *The effect of thyroid hormone with and without the standard dose of cortisone on the survival time of rats allowed unlimited access to food*

The purpose of this experiment was to study the effect of the thyroid hormone and of the standard dose of cortisone on the survival time of rats allowed to eat *ad lib.* The results are summarised in Table 1. It can be seen that the average survival time of the control animals on the necrogenic diet is 30.00 ± 2.6 days. Death in all animals of this group was caused by the rather sudden onset of hepatic necrosis, the appearance of which ranged from an acute "haemorrhagic" stage to a more protracted form. In agreement with the findings of Abell and Beveridge (9), it was found that the ingestion of thyroid powder significantly ($P < 0.001$) hastened the onset of hepatic necrosis (17 ± 1.4 days), the appearance of which did not differ from that of the control group. The standard dose of cortisone, on the other hand, almost doubled ($P < 0.001$) the average survival time of rats so treated, (58.23 ± 6.4 days). This favourable effect of cortisone, first observed by Schwarz (10), was seen almost in every animal so treated, but its extent varied greatly. Two out of 13 animals failed to develop liver necrosis; in the remainder the necrosis appeared to take a more protracted course and seemed more extensive than in the control groups, but otherwise no significant difference was observed. In contrast to the favourable effect

[3] Total iodine content: about 0.216%; in combination as thyroxine: 0.105%.

TABLE 1. THE EFFECT OF CORTISONE (STANDARD DOSE: 1 MG./100 GM. BODY WEIGHT) AND OF THYROID HORMONE (0.2%) ON THE SURVIVAL TIME (IN DAYS) OF RATS FED THE "NECROGENIC" DIET. (FOOD INTAKE NOT LIMITED)

No. of animals per treatment group	"Necrogenic" diet	"Necrogenic" diet plus thyroid powder (0.2%)	"Necrogenic" diet plus cortisone (1mg./100 gm. b.w.)	"Necrogenic" diet plus thyroid powder (0.2%) plus Cortisone (1 mg./100 gm.)
13	30.0±2.6	17.3±1.4	58.2±6.4	19.8±1.0

of cortisone in this group the simultaneous administration of thyroid powder completely nullified the delaying effect of this steroid, so that the mean survival time of rats given cortisone and thyroid hormone together (19.8 ±1.0 days) did not significantly differ from that of rats given thyroid powder alone (17.3±1.4 days); neither did the incidence or appearance of hepatic necrosis. It could, therefore, be concluded that the favourable effect of cortisone could be abolished by the production of a hyperthyroid state or, alternatively, that cortisone, in the dose used here, had no effect on the course of the hyperthyroid state.

Experiment II. *The effect of thyroid hormone with and without the standard dose of cortisone on the survival time of rats with restricted food intake*

Unpublished observations as well as a study of the food intake of the animals of the present series had shown that rats treated with cortisone tended to eat more than their controls. Conceivably, therefore, a higher degree of hyperthyroidism had been induced in the rats injected with cortisone and allowed an unlimited intake of the diet containing thyroid hormone than in those animals fed that diet alone. These 2 groups of Experiment I are therefore not strictly comparable, and it was considered important to eliminate the question of unequal food intake as a potential source of error by restricting the amount of food eaten in the manner already described in the methods used. The results of this experiment are shown in Table 2. With the exception of a more pronounced effect of cortisone in animals given the basic diet the results are similar to those obtained in the previous experiment; above all, despite the restriction of food intake, the mean survival time of rats treated with thyroid hormone with and without cortisone did not differ greatly. Detailed comparison of the individual survival times of the corresponding littermates, however,

TABLE 2. THE EFFECT OF CORTISONE (STANDARD DOSE) AND OF THYROID HORMONE ON THE SURVIVAL TIME (IN DAYS) OF RATS FED THE "NECROGENIC" DIET. (FOOD INTAKE RESTRICTED)

No. of animals per treatment group	"Necrogenic" diet	"Necrogenic" diet plus thyroid powder (0.2%)	"Necrogenic" diet plus cortisone (1mg./100 gm. b.w.)	"Necrogenic" diet plus thyroid powder (0.2%) plus cortison
10	32.0±3.6	18.6±1.0	94.0±11.2	23.0±1.6

showed that in 7 out of 10 rats treated with cortisone and fed the diet containing thyroid powder the survival time was longer, in 1 identical with, and in 2 shorter than that of the littermate fed the same diet, but not given cortisone. This distinct trend towards the prolongation of the survival time of the hyperthyroid animals under the influence of cortisone suggested the next experiment in which the effect of a larger dose of cortisone was investigated.

Experiment III. *The effect of an increased dose of cortisone on the survival time of hyperthyroid rats with restricted food intake*

In this experiment the effect of varying doses of cortisone on animals with the same degree of hyperthyroidism was studied. To this purpose the leading animal, given only the diet containing the thyroid powder, was

TABLE 3. THE EFFECT OF DOUBLING THE STANDARD DOSE OF CORTISONE ON THE SURVIVAL TIME (IN DAYS) OF HYPERTHYROID RATS FED THE "NECROGENIC" DIET. (FOOD INTAKE RESTRICTED)

No. of animals per treatment group	"Necrogenic" diet plus thyroid powder (0.2%)	"Necrogenic" diet plus thyroid powder plus cortisone (1mg./100 gm. b.w.)	"Necrogenic" diet plus thyroid powder plus cortisone (2mg./100 gm. b.w.)	"Necrogenic" diet
12	19.7±1.2	25.8±2.0	31.6±2.4	32.8±1.7

again allowed to eat as much as it liked, but the second and third littermate, whose intake of the thyroid diet was appropriately restricted, were in addition treated with 1 mg, respectively 2 mg., of cortisone per 100 gm. body weight. The fourth animal served as untreated control given restricted amounts of the basic diet only. Table 3 shows that the early onset of death from liver necrosis caused by the ingestion of thyroid hormone was slightly, but definitely, postponed by the standard dose of cortisone; the effect of doubling the dose of cortisone was a further significant increase in the survival time of the hyperthyroid animals, which now lived as long as their untreated controls fed the basic diet only. Statistical analysis of the data led to the conclusion that there was a highly significant relationship between the dose of cortisone and the survival time of animals treated with thyroid powder; the increase in survival time was proportional to the dose of cortisone. In other words: the deleterious effect of the hyperthyroid state could be counteracted to a significant extent by treatment with an adequate amount of cortisone.

Experiment IV. *The effect of high doses of cortisone on the survival time of hyperthyroid rats allowed unlimited access to food*

The significant increase in the survival time obtained by doubling the standard dose of cortisone described in the preceding experiment suggested

TABLE 4. THE EFFECT OF A "TOXIC" DOSE OF CORTISONE (5 MG./100 GM. BODY WEIGHT) ON THE SURVIVAL TIME (IN DAYS) OF HYPERTHYROID RATS FED THE "NECROGENIC" DIET. (FOOD INTAKE NOT LIMITED)

No. of animals per treatment group	"Necrogenic" diet	"Necrogenic" diet plus thyroid powder (0.2%)	"Necrogenic" diet plus cortisone (5mg./100 gm. b.w.)	"Necrogenic" diet plus thyroid powder (0.2%) plus cortisone (5mg./100 gm. b.w.)
8	23.9±1.7	15.6±0.7	25.9±3.3	21.6±2.7

a study of the effect of still higher doses of that hormone. The experiment presented here is similar in its arrangement to the first experiment described, from which it differs only in the dose of cortisone used; instead of the standard dose of 1 mg., a daily dose of 5 mg. cortisone acetate per 100 gm. body weight was given. The results are shown in Table 4, and will be described in greater detail at a later date. Here it is sufficient to state that cortisone in these large doses no longer exerted the favourable effect seen with the standard dose. Most animals died either earlier or at about the same time as their controls not treated with cortisone; judging by their appearance and by the decrease in their food intake the excess of cortisone given seemed to exert a "toxic" effect. This interpretation is in keeping with the fact that their cause of death could not be attributed to liver necrosis, which had not occurred in 6 out of 8 rats of this group. For the purpose of this paper it is important to stress that the "toxic" effects of these large doses of cortisone were not influenced or counteracted by the degree of hyperthyroidism induced, but "t" tests suggested that, by comparison with Experiments I and II, there appeared to be a reversal of the interaction between cortisone and the thyroid hormone in the sense that in the rats treated with thyroid powder cortisone seemed to have a relatively greater effect.

Experiment V. *The effect of thyroid hormone on the effect of ACTH*

The results of the preceding experiments raised the question whether the antagonism observed between the effect of the thyroid hormone and of extraneous cortisone applied equally to the secretion of the rat's own adrenal cortex. It can be seen from Table 5 that treatment with ACTH, even if started as late as on the 20th day of the experiment, significantly prolonged the survival of the rats on the deficient basic diet. Yet, as in the

TABLE 5. THE EFFECT OF THYROID HORMONE ON THE SURVIVAL TIME OF RATS TREATED WITH ACTH AFTER 19 DAYS ON THE "NECROGENIC" DIET. (FOOD INTAKE NOT RESTRICTED)

No. of animals per treatment group	"Necrogenic" diet	"Necrogenic" diet plus ACTH	"Necrogenic" diet plus thyroid powder (0.2%) plus ACTH
7	26.0±2.4	57.8±6.7	27.1±1.5

corresponding experiments with cortisone, the simultaneous induction of a hyperthyroid state completely prevented this favourable effect of ACTH.

DISCUSSION

From the experiments presented here it is apparent not only that cortisone and the thyroid hormone, when given separately, have opposite effects on the survival times of experimental animals fed the deficient diet causing liver necrosis, but also that these effects can cancel each other out when the hormones are given together. Not only can the induction of a hyperthyroid state completely obliterate some of the manifestations of an excess of cortisone in the body but, conversely, the administration of an adequate amount of cortisone can, to a certain extent, oppose the deleterious course of hyperthyroidism as well. In addition, the experiments here have shown how important the quantitative aspect of the thyroid-adrenal relationship is. By merely raising the amount of cortisone given to the animals with approximately the same degree of hyperthyroidism it was possible to obtain responses which ranged from "no effect of cortisone" to a "favourable" or even "toxic" effect. Conversely, a dose of cortisone which had a marked effect in the euthyroid rat had little or no effect in the hyperthyroid rat. These findings are in agreement with the view that the increased metabolic requirements of the hyperthyroid organism lead to increased demands for, and "utilisation" and destruction of, adrenocortic a steroids so that a dose of extraneous cortisone which produces signs of hypercorticism in the hypo- or euthyroid animal will no longer be adequate even to cover the "basic" requirements of the hyperthyroid rat.

An interesting conclusion can be drawn from Experiment V in which the simultaneous administration of ACTH and thyroid powder has been studied. Had the previous experiments not shown that a dose of injected cortisone which was highly effective in the euthyroid rat was inadequate in the hyperthyroid animal, it would have been possible to conclude that the lack of effect seen after treatment with ACTH in the hyperthyroid rat was due to the decreased responsiveness of the adrenal cortex to its stimulating hormone, analogous to the change in sensitivity of end organs which is assumed by some workers (11, 12), to occur in the hyperthyroid state. The experiments presented here suggest an alternative explanation: The adrenal cortex is fully responsive to the injected ACTH, but the steroids produced are more rapidly metabolised, and therefore less likely to reach the level required for the characteristic steroid effects. An additional factor has to be considered. There is no reason to assume that the more rapid turnover postulated here is limited only to the adrenocortical steroids; it could equally well apply to the injected ACTH itself so that, conceivably, for purely quantitative reasons the adrenal cortex of the hyperthyroid animal was less stimulated, but not necessarily less responsive, than that of its euthyroid control. The same reasoning could, of course, be applied to other

instances of "lessened responsiveness" of a target organ to its stimulating hormone in the hyperthyroid organism, or, in its reverse formulation, to those instances where an increase in the sensitivity of an end organ has been assumed to be caused by the induction of a hypothyroid state (13).

SUMMARY

The effect of thyroid hormone and of cortisone or ACTH, given either singly or combined, on the survival time of weanling rats fed a diet deficient in sulphur-containing amino-acids and tocopherol was studied. In control animals this diet invariably produced liver necrosis; thyroid hormone significantly shortened, cortisone or ACTH significantly lengthened the survival time. If given together, the effects of these hormones tended to oppose each other. A dose of cortisone which was highly effective in the euthyroid animal had no effect in the hyperthyroid rat. If, however, the amount of cortisone given was increased, the deleterious effect of the hyperthyroid state was significantly inhibited, so that the survival time of the hyperthyroid rat now equalled that of the untreated control. These findings are interpreted in the light of the hypothesis that the hyperthyroid state leads to an increased demand for, and "utilisation" of, adrenocortical steroids. The significant lack of effect of ACTH in the hyperthyroid rat, as opposed to its euthyroid control, has been interpreted on similar lines, and suggests that it may be necessary to revise current views on the "sensitivity" of an end organ to its stimulating hormone in the hyperthyroid organism.

Acknowledgments

I should like to thank Dr. D. Darlington for the statistical analysis of the data, and Miss D. King for her technical assistance.

Gifts of thyroid powder from Parke, Davis & Co., cortisone acetate from Merck & Co., "Cortrophin Z" from Organon Ltd., and Vitamins from Roche Ltd., are gratefully acknowledged.

REFERENCES

1. Aterman, K.: *Lancet* **1**: 1143. 1952.
2. Aterman, K.: *Acta Endocrinol.* **15**: 1954. Suppl. XX.
3. Aterman, K. and S. M. Greenberg: *A.M.A. Arch. Ophth.* **51**: 822. 1954.
4. Greenberg, S. M. and K. Aterman: *Metabolism* **4**: 264. 1955.
5. Levin, M. E. and W. H. Daughaday: *J. Clin. Endocrinol. & Met.* **15**: 1499. 1955.
6. Peterson, R. E., J. B. Wyngaarden, S. L. Guerra, B. B. Brodie and J. J. Bunim: *J. Clin. Endocrinol. & Met.* **34**: 1779. 1955.
7. Peterson, R. E. and J. B. Wyngaarden: *J. Clin. Invest.* **35**: 552. 1956.
8. Lindan, O. and H. P. Himsworth: *Brit. J. Exp. Path.* **31**: 651. 1950.
9. Abell, M. R. and J. M. R. Beveridge: *A.M.A. Arch. Path.* **52**: 423. 1951.
10. Schwarz, K.: *Science* **113**: 485. 1951.
11. Smelser, G. K.: *Anat. Rec.* **73**: 273. 1939.
12. Warner, E. D. and R. K. Meyer: *Endocrinology.* **45**: 33. 1949.
13. Hess, M.: *Proc. Soc. Exp. Biol. & Med.* **84**: 127. 1953.

EFFECT OF ANTIGONADOTROPHIC SERUM ON POST-IRRADIATION CHANGES IN THE REPRODUCTIVE SYSTEM OF FEMALE MICE[1]

CHARLES A. ELY[2]

Department of Anatomy, College of Physicians and Surgeons, Columbia University, New York, New York

COMPLETE alteration of the microscopic appearance of the ovary follows exposure of the adult mouse to x-rays. Primitive ova disappear within a few days, and the more mature ova and the granulosa cells associated with them are gradually replaced by hypertrophied luteinized cells of considerable structural variability and of doubtful origin (1, 2). Subsequent gradual changes lead to the appearance of neoplastic growths of several kinds in 7 to 12 months (3, 4).

It has been suggested that the impetus for this series of events is provided by increased secretion of gonadotrophic hormones which occurs as a consequence of diminished production of estrogen after irradiation (5, 6, 7). The following pages describe the modifications in this sequence of changes in the ovary induced by the injection of an antigonadotrophic serum after irradiation.

MATERIALS AND METHODS

Swiss mice[3] 50 to 60 days-of-age were given a single dose of 175r[4]. At intervals after irradiation (See Table 1), injections of antigonadotrophic serum or control materials were begun. Two-tenths ml. of all solutions was injected subcutaneously daily 5 days each week for 6 weeks.

The antigonadotrophic serum was developed in rabbits after prolonged injection of crude sheep pituitary extract prepared according to the method of McShan and Meyer (8). The extract has been characterized, and the method of assay of the antigonadotrophic serum described elsewhere (9, 10). Samples from the same pooled antihormone serum were used in 4 groups receiving the first injection 0, 1, 2, and 4 weeks after irradiation. The remaining 3 groups received antisera from several different sources. In any case, only sera which showed strong antigonadotrophic activity were used. Control ma-

Received September 12, 1956.

[1] Aided by grants from the Anna Fuller Fund and the Damon Runyon Memorial Fund for Cancer Research.

[2] The technical assistance of Edith Reismann and Rosemarie Tuercke has made this report possible.

[3] Obtained from Manor Farms, Staatsburg, New York.

[4] The author wishes to thank Dr. Roberts Rugh of the Radiological Research Laboratory of Columbia University for supervising the irradiation of the animals used in this study.

The physical factors of irradiation were: 210 KVP, 15 ma, 0.28 mm. Cu and 0.50 mm. Al filter, focus middle body distance 50 cm., output 97.7 r/min in air.

TABLE 1. EFFECT OF ANTIGONADOTROPHIC SERUM ON BODY AND ORGAN WEIGHTS OF X-RAYED MICE

Weeks after x-ray injection begun	Days x-ray to autopsy	Treatment	Number animals	Body weight (gm.)	Ovarian weight or volume (mg., mm.³)[1]	Uterine weight (mg.)	Spleen weight	Thymus weight (mg.)
0	40	AS[2]	8	33.2	2.9±1.1[7]	29.5± 3.7[7]	258.9	62.8
	40	ASH[3]	3	31.7	7.4±3.4	98.7±33.8	203.7	56.3
	40	SAL[4]	3	31.0	12.9±4.9	120.5±43.3	163.7	64.3
1	45–50	AS	15	31.9	4.1±2.0	37.8±19.6	232.8	65.8
	45–50	NS[5]	6	28.0	7.4±1.3	60.4± 7.2	378.6	20.8[9]
	45–50	ASH	6	31.3	8.0±4.4	84.5±42.4	218.8	57.7
	45–50	SAL	6	30.0	9.7±5.8	114.1±75.2	178.5	61.3
2	50–55	AS	8	34.5	4.0±1.3	35.8± 6.0	303.3	64.8
	50–55	ASH	3	30.6	12.0±5.8	77.7±15.8	255.9	61.9
	50–55	SAL	3	32.1	14.4±3.8	126.5±44.1	188.7	63.3
4	65	AS	7	33.7[6]	3.5±0.8	34.8± 7.1	295.3	59.5
	65	NS	5	30.6	6.5±2.1	65.8±44.3	234.2	80.8
	65	ASH	3	30.2	5.3±1.6	82.7±41.0	217.4	53.1
	65	SAL	3	29.1	9.0±7.6	91.2±35.6	155.9	62.7
8	100	AS	6	32.5	7.2±1.3[8]	40.9±10.0	396.2	67.2[6]
	100	NS	5	32.8	8.8±3.3	94.1±23.6	463.2	44.7
	100	ASH	2	32.8	9.3±2.7	85.8±15.0	197.4	55.3
	100	SAL	3	30.1	9.4±2.2	120.7±15.0	169.3	53.6
16	155–165	AS	7	35.4	1.6±1.0	38.2± 7.5	482.6[6]	32.0
	155–165	NS	2	32.2	7.1±4.4	101.8±37.3	354.6	32.7
	155–165	ASH	3	35.0	7.1±5.7	61.1±23.7	251.7	41.9
	155–165	SAL	3	30.1	5.8±2.5	142.9±44.4	161.6	53.2
24	220–225	AS	9	34.8	2.1±1.2	39.3±12.1	302.2	37.6
	200–225	NS	6	34.4	7.7±6.8	71.9±23.8	338.4	39.7
	200–225	ASH	4	39.1	5.6±5.2	90.6±30.2	366.8	71.6
	200–225	SAL	6	31.6	7.8±5.4	111.9±34.0	143.3	35.2

[1] Values 0–4 weeks after x-ray in mm.³; 8–24 weeks in mg.
[2] Antigonadotrophic serum.
[3] Sheep heart antiserum.
[4] Saline.
[5] Normal rabbit serum.
[6] Body, spleen, and thymus weights of animals which received antigonadotrophic serum were compared statistically with pooled serum controls; except where indicated the differences were not significant.
[7] Mean ±Standard Deviation.
[8] Ovarian weights (or volumes) and uterine weights of animals which received antigonadotrophic serum were compared statistically with pooled serum controls; except where indicated the differences were significant.
[9] Significantly different when compared with other groups injected one week after x-ray.

terials included normal rabbit serum, saline, and sheep heart antiserum. The latter was developed in rabbits by the injection of an extract of sheep hearts prepared by following the same procedures as those used in extraction of pituitary glands. Subsequently, the sheep heart extract was handled similarly to the pituitary extract. Antibodies to pituiary extract and to the sheep heart extract were demonstrated in a representative sample of each antiserum by the agar-plate method of Ouchterlony (11).[5]

[5] Dr. Beatrice Seegal of the Department of Microbiology kindly made available her time and that of her staff as well as laboratory facilities for carrying out the immunological procedures.

At the end of 6 weeks (after 30 injections), autopsies were performed. The ovaries,[6] uterus, thymus, and spleen were weighed. One ovary, a segment of one horn of the uterus, the kidneys, adrenals, and pituitary glands were fixed in Bouin's solution. Interrupted serial sections of the ovaries, pituitary, and adrenal glands were cut at 5μ from paraffin blocks. Sections of each of these tissues and of kidney and uterus were stained by the hematoxylin and eosin and the periodic acid-Schiff method.

Vaginal smears of some animals in each group were taken daily except Sunday during the period of injection.

RESULTS

Data presented in Table 1 show that the ovaries and uteri of x-rayed animals which received antigonadotrophic serum were smaller than those of controls. With exception of the ovaries of the group injected 8 weeks after irradiation, the differences were statistically significant when pooled controls were compared with antihormone injected animals.

A quantitative appraisal of the intensity of the gonadotrophic stimulation is given in Table 2. Estimates were based upon the degree of develop-

[6] The ovaries in the groups injected 1–4 weeks after irradiation were not weighed. Instead, after serial sectioning, volumes were determined by planimetric measurement.

TABLE 2. EFFECT OF ANTIGONADOTROPHIC SERUM ON THE HISTOLOGICAL APPEARANCE OF THE OVARY OF X-RAYED MICE

Weeks after x-ray injection begun	Days x-ray to autopsy	Treatment	Number ovaries	I Hypertrophied[1] cells					II "Deficiency"[2] cells					III Tubular[3] down-growths			
				+++	++	+	−	0	+++	++	+	−	0	+++	++	+	−
0	40	AS[4]	10	0	0	0	6	4	0	4	2	0	4	0	0	0	10
	40	Cont.[5]	6	3	2	1	0	0	0	0	1	5	0	0	0	0	6
1	45–50	AS	14	0	0	7	7	0	3	4	0	7	0	0	0	0	14
	45–50	Cont.	18	7	7	4	0	0	0	0	2	16	0	0	0	0	18
2	50–55	AS	10	0	0	1	8	1	1	7	1	0	1	0	0	0	10
	50–55	Cont.	6	4	2	0	0	0	0	0	0	6	0	0	0	0	6
4	65	AS	9	0	0	0	9	0	3	6	0	0	0	0	0	0	9
	65	Cont.	13	1	7	4	0	1	0	1	3	8	1	0	0	0	13
8	100	AS	6	1	5	0	0	0	0	0	1	5	0	0	0	0	6
	100	Cont.	12	7	1	3	0	1	0	0	0	11	1	0	0	1	11
16	155–165	AS	9	1	0	0	0	8	0	0	0	1	8	0	0	0	9
	155–165	Cont.	10	5	3	1	0	1	0	0	0	9	1	0	0	7	3
24	200–225	AS	12	1	0	3	4	4	1	2	2	3	4	0	1	8	3
	200–225	Cont.	23	8	3	8	3	1	0	4	4	14	1	8	6	5	4

[1] +++ Ovaries composed of hypertrophied interfollicular cells with abundant granular cytoplasm and smoothly rounded nuclei. (See Fig. 2.)
++ Ovaries composed of hypertrophied interfollicular cells, but with areas of loosely arranged disorganized interfollicular cells deficient in cytoplasmic granules, with increased cytoplasmic vacuolization, and with nuclei of irregular outline exhibiting varying degrees of pyknosis.
+ Ovaries similar to above, but with characteristics exaggerated; only a few typical hypertrophied cells.
− Ovaries lacking hypertrophied cells, composed of interfollicular cells in various stages of degeneration.
0 Ovaries composed largely of fibrous connective tissue with a few degenerate interfollicular cells and "brown cells of degeneration." (See Figs. 4, 5.)

[2] +++, ++, + Ovaries with many, several, or few "deficiency" cells. (See Fig. 10.)
0 Ovaries composed largely of fibrous connective tissue with a few degenerate interfollicular cells and "brown cells of degeneration." (See Figs. 4, 5.)

[3] +++ Ovaries in which tubular down-growths occupied a large part of the ovarian cortex.
++ Ovaries in which limited areas were made up of tubular down-growths.
+ Ovaries with a few isolated tubular down-growths.
− Ovaries lacking any tubular down-growths.

[4] Antigonadotrophic serum.

[5] Control. Includes animals receiving normal rabbit serum, sheep heart antiserum, or saline.

ment and apparent viability of hypertrophied interfollicular cells[7] and on the prevalence of "deficiency" cells (See below).

Interfollicular cells regarded as fully stimulated were clearly enlarged and of polyhedral shape. The granular cytoplasm was abundant, and cell walls were evident. The nuclei were smoothly rounded. They resembled lutein cells to a greater or less degree (Figs. 1, 2). These cells predominated in the ovaries of control animals (Table 2, Column I). Eighty-two per cent of the ovaries having hypertrophied interfollicular cells were found among the controls, whereas 90% of the ovaries in which hypertrophied cells were lacking were treated with antigonadotrophic serum. As was the case when ovarian weights were used as the basis for comparison, a possible exception was noted in the distribution of hypertrophied interfollicular cells in the group injected 8 weeks after irradiation, although, even here, the tendency was the same.

The "deficiency" cells resembled those found in the rat ovary after hypophysectomy which were described by Selye *et al.* (12). They were characterized by a round or oval hyperchromatic nucleus with heavy chromatin granules often arranged around the periphery. The walls of these cells were not visible and the cytoplasm was scanty (Fig. 10). Seventy-one per cent of all the ovaries in which "deficiency" cells were found had received antigonadotrophic serum. Their absence from ovaries of control animals was the rule (Table 2, Column II). A few ovaries, found largely among the antihormone treated animals, appeared to be totally degenerate consisting of fibrous tissue, degenerate interfollicular cells, and "brown cells of degeneration" (Figs. 3, 4, 5; "0" Table 2).

The first evidence of tubular down-growths from the germinal epithelium was encountered 150 days after irradiation, which is in accord with the observation of Butterworth (4). At this time they were not numerous, but at 200 days after irradiation they had become a conspicuous part of many ovaries. Proliferation of these characteristic structures appeared to have progressed further in the controls than in the animals which were treated with antigonadotrophic serum (See Table 2, Column III).

Uteri of antihormone treated animals differed markedly from those of controls. In the former, all the layers were reduced in thickness. The epithelium was low, and the endometrial stroma was dense with spindle-shaped nuclei predominating among the interstitial cells. The uterine glands were smaller and fewer in number in antihormone treated organs (Figs. 9, 11). In the uteri of controls the epithelium was high, the stroma edematous, and the uterine glands were enlarged and numerous. Cases in which enlarged vesicular stromal nuclei were abundant were occasionally encountered (Figs. 7, 8).

[7] Refers to lutein-like cells which constituted the bulk of the irradiated ovaries and which were not clearly associated with a recognizable follicle or corpus luteum.

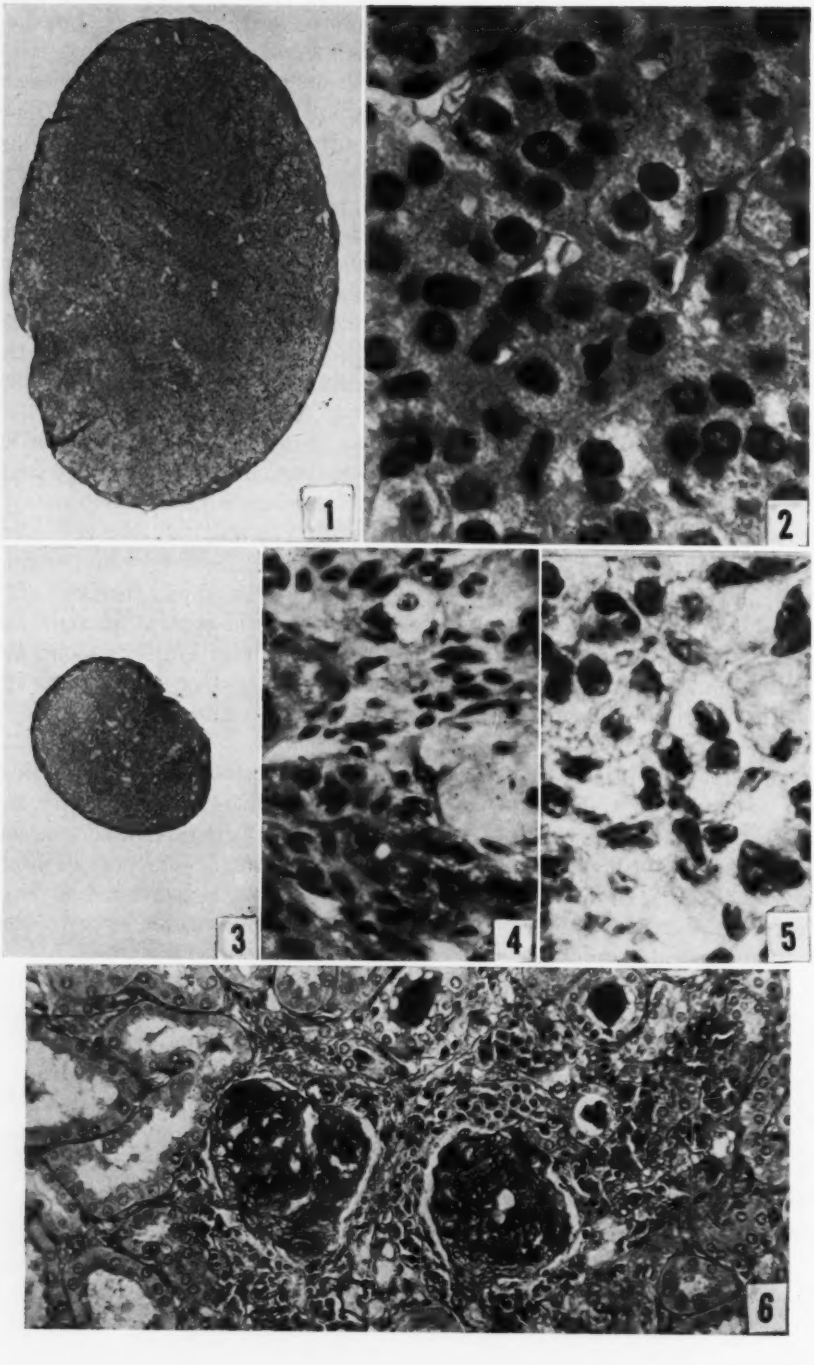

1
2
3
4
5
6

TABLE 3. EFFECT OF ANTIGONADOTROPHIC SERUM ON THE VAGINAL SMEAR OF X-RAYED MICE

	Treatment	Weeks after x-ray, injection begun						
		0	1	2	4	8	16	24
Total number of days smeared[1] (average per mouse)	AS[2]	39	40	37	40	34	32	22
	Cont.[3]	37	40	38	38	34	30	27
Number of days with cornified cells (average per mouse)	AS	1(8)[4]	2(7)	1(8)	2(8)	3(7)	1(8)	1(14)
	Cont.	8(12)	8(11)	8(12)	8(12)	13(11)	11(12)	1(18)

[1] Vaginal smears taken during the injection period.
[2] Antigonadotrophic serum.
[3] Control. Includes animals receiving normal rabbit serum, sheep heart antiserum, or saline.
[4] Numbers in parentheses indicate number of animals.

Injection of antigonadotrophic serum decreased the frequency of vaginal smears in which cornified cells were predominant (Table 3). Typical animals receiving antihormone did not show evidence of vaginal cycling after the first or second week. Control animals usually completed 4 or 5 cycles during a period of 40 days when injections were being given. The fact that few animals injected 24 weeks after irradiation had cornified vaginal smears, regardless of the nature of the injected material, is in accord with the observation of Geist *et al.* (13). It may be noted here that the fluid distended uterus typical of the proestrous phase of the vaginal cycle was not encountered.

EXPLANATION OF PLATES

All sections were stained with hematoxylin and eosin except where otherwise indicated.

FIG. 1. Ovary. ×30. Saline injections begun 16 weeks after irradiation. This ovary was designated "+++" in Column I of Table 2.

FIG. 2. Ovary. ×950. Sheep heart antiserum injections begun 16 weeks after irradiation. Both ovaries shown in Fig. 1 and 2 were made up entirely of large hypertrophied interfollicular cells with cytoplasm packed with eosinophilic granules. This ovary was designated "+++" in Column I of Table 2.

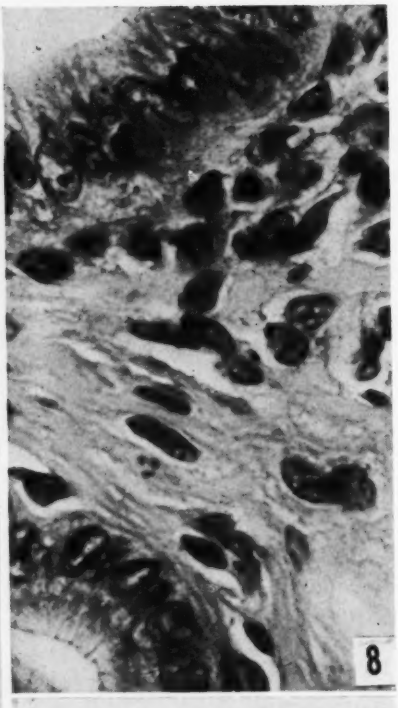
FIG. 3. Ovary. ×30. Antihormone injections begun 16 weeks after irradiation. Much of this gland was a confused mixture of collagenous tissue, large interfollicular cells in various stages of degeneration, and "brown cells of degeneration." This ovary was designated "0" in Table 2.

FIG. 4. Ovary. ×950. Same as in Figure 3. From an area showing "brown degeneration" (at right), collagenous tissue (throughout), and degenerate large interfollicular cells.

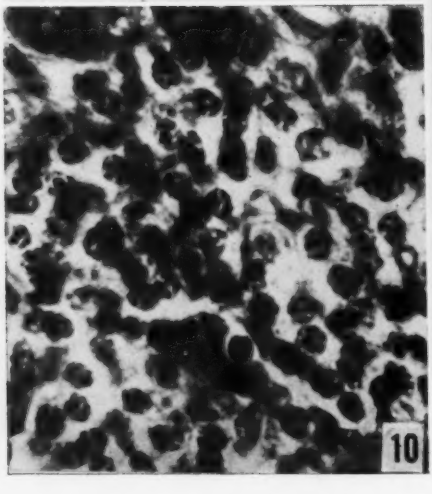
FIG. 5. Ovary. ×950. Same as in Figure 3. From an area showing degenerate large interfollicular cells.

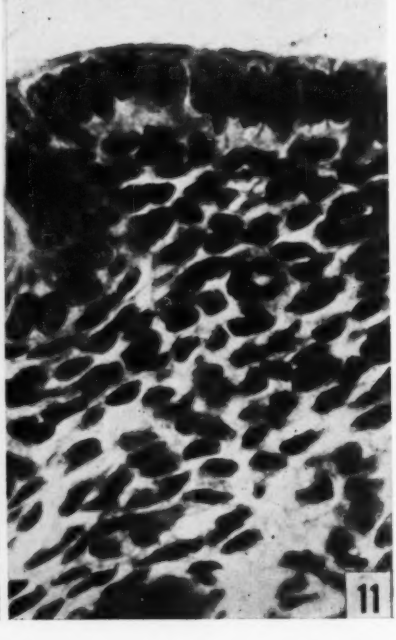
FIG. 6. Kidney. ×280. Normal serum injections were begun 24 weeks after irradiation. Two abnormal glomeruli are shown. Increased thickness of the parietal layer of Bowman's capsule can be seen in both. Casts of Schiff-positive material are shown in 3 tubules. Periodic acid-Schiff stain.

7


8

9


10


11

The adrenal glands of all animals were examined. No abnormalities were found which could be correlated with experimental procedures. Histological preparations of pituitary glands were studied, but differential cell counts were not undertaken. However, it seemed certain that there was an increase in the total number of basophils in the antihormone-treated animals of all experimental groups[8] studied. Large pale blue basophils with finely granulated or foamy cytoplasm were found aggregated at the periphery of the gland. In control animals small evenly distributed cells with coarse periodic acid-Schiff positive granules were frequently encountered.

With the exception noted in Table 1, the effect, or lack of effect, of injections upon the body weight or upon the weight of the spleen or thymus was consistent. Distinctions based upon the type of serum injected were not possible.

A mild subcutaneous edema developed in a number of serum-treated animals after 2 to 3 weeks of injection. It was followed by the appearance of subcutaneous lesions in which heavy deposits of caseous material occurred; these animals were discarded. Because of the similarity of this reaction to the Arthus phenomenon, a skin hypersensitive reaction induced in some laboratory animals and man by the injection of foreign protein (14), and because of the suspected association of hypersensitivity with the pathogenesis of kidney lesions (15), kidney sections from all animals were examined.

Lesions[9] involving the glomerulus were observed in 30 to 50% of the kidneys from animals which received serum 8 to 24 weeks after irradiation

[8] Due to accidental loss no pituitary glands from animals injected 16 weeks after irradiation were available.

[9] The author is indebted to Dr. Thelma B. Dunn of the National Institute of Health and to Dr. Margaret Bebans of the Columbia Research Service, Goldwater Memorial Hospital who examined some of the kidney material and identified the lesion. The author, however, is responsible for the conclusions drawn.

FIG. 7. Uterus. ×30. Same as in Figure 2. Thickness of all layers is evident when compared with Figure 9.

FIG. 8. Uterus. ×950. Same as in Figure 2. Estrogenic stimulation is seen in the heightened epithelium of the lumen, edematous stroma, and enlarged glands (lower left). Progestational effects are evident from the characteristically enlarged vesicular stromal nuclei with a conspicuous nucleolus.

FIG. 9. Uterus. ×30. Antihormone injections begun 16 weeks after irradiation. Reduced thickness of all layers is evident.

FIG. 10. Ovary. ×950. Antihormone injections begun 24 weeks after irradiation. "Wheel" or "deficiency" cells indicate lack of gonadotrophic stimulation. This ovary was designated "+++" in Column II of Table 2. Periodic acid-Schiff stain.

FIG. 11. Uterus. ×950. Same as in Figure 9. The low epithelium, dense stroma, and small dark, fusiform stromal nuclei indicate little or no stimulation by estrogen or progesterone.

(Fig. 6). None was encountered in the younger animals. Lesions were distributed uniformly among the serum-treated animals. It was not possible to demonstrate correlation between the presence of glomerular lesions and the type of serum injected, or between the degree of response of the ovary and the presence of renal lesions.

DISCUSSION

It is evident that gonadotrophic activity was eliminated or greatly reduced. With a single exception, the reduction in weight or volume of the ovaries after injection of antihormones was substantial. As well, histological differences between ovaries of control groups and antihormone treated groups were clear and distinct. The presence of "deficiency" cells consistently in the ovaries of animals which received antigonadotrophic serum points to the similarity between the effect of the antigonadotrophic serum treatment and hypophysectomy.

Further lack of hormonal activity was seen in the weight and histological appearance of the uteri. The lack of estrogenic activity is attested to by the lower epithelial cells, the dense compact condition of the endometrial stroma, and the small size and number of the uterine glands. In effect, when the criteria of Hooker and Forbes (16) were applied it was clear that estrogenic and progestational stimulation was at a minimum in the animals which had been given antihormone serum.

The ease with which function of the pituitary gland and, indirectly, that of the reproductive system may be impaired is well known (17). For this reason, results suggesting specific deleterious effects of an experimental manipulation upon the reproductive system must be evaluated accordingly.

On the assumption that they would share many of the general properties of the antigonadotrophic serum, sheep heart antiserum and normal rabbit serum were used as control materials in addition to saline. Data presented in Table 1 leave no doubt that either sheep heart antiserum or normal rabbit serum reduces the size of the reproductive organs to some extent. However, the differences between antihormone injected animals and controls were significant whether the comparison was made with all controls considered as a group, or with only the normal serum and sheep heart antiserum controls combined. Had the effect of antigonadotrophic serum been nonspecific, consistent differences in the response of the thymus glands, spleen, and body weight would have been expected. This was not the case (see Table 1).

In conjunction with the present study, some observations were made of antigonadotrophic serum effects in nonirradiated animals. In general normal adult animals did not show the same reduction in weight of the reproductive organs, and the vaginal cycles were not so strikingly altered after

treatment with antigonadotrophic serum. Whether these differences have a quantitative basis or otherwise has not been determined.

The possibility that the antigonadotrophic serum may alter kidney function cannot be entirely ruled out. But, the fact that recognizable kidney lesions were distributed without reference to the type of serum injected does not tend to implicate the kidneys.

That irradiation may induce, in some way, a hormonal imbalance which enhances the efficacy of the antigonadotrophic serum is possible. It is, however, well known that, with the exception of the gonads, other endocrine organs are singularly resistant to x-rays (18).

Data are lacking which would permit a conclusive statement regarding the apparent inhibition of the development of tubular down-growth from the germinal epithelium. Hitherto, they have been considered to be independent of hormonal influence (19), but the results observed suggest the contrary. Further, experiments may reveal an early, but rapidly divested, dependence of the down-growths from the germinal epithelium upon gonadotrophic stimulation.

SUMMARY

An antigonadotrophic serum produced in rabbits by prolonged injection of a crude sheep pituitary extract was given to young adult Swiss female mice at intervals ranging from 0 to 24 weeks after total-body irradiation. The well known changes which occur in the ovary of the mouse after irradiation were greatly modified. Ovaries of mice receiving injections of antihormone were smaller in weight or volume than those of animals treated with appropriate control materials, and the hypertrophied lutein-like cells which characterize the ovaries of irradiated mice were missing or showed conspicuous degenerative changes. Lack of or reduced gonadotrophic stimulation was also reflected in the weight and histological appearance of uteri of antihormone injected animals. With the possible exception of the pituitary gland, no alterations were observed in other viscera or in body weights which could be specifically ascribed to the antigonadotrophic serum.

REFERENCES

1. Brambell, F. W. R. and A. S. Parkes: *Proc. Roy. Soc.*, London, s.B, **101**: 316. 1927.
2. Murray, J. M.: *Am. J. Roentgenol.* **25**: 1. 1931.
3. Furth, J. and J. S. Butterworth: *Am. J. Cancer* **28**: 66. 1936.
4. Butterworth, J. S.: *Am. J. Cancer* **31**: 85. 1937.
5. Li, M. H., W. U. Gardner and H. S. Kaplan: *J. Nat. Cancer Inst.* **8**: 91. 1947.
6. Furth, J. and M. C. Boone: *Cancer Res.* **7**: 241. 1947.
7. Lick, L., A. Kirschbaum and H. Mixer: *Cancer Res.* **9**: 532. 1949.
8. McShan, W. H. and R. K. Meyer: *J. Biol. Chem.* **151**: 259. 1943.
9. Ely, C. A.: *Endocrinology*: **59**: 83. 1956.
10. Ely, C. A.: *Proc. Soc. Exp. Biol. and Med.* **84**: 501. 1953.

11. Ouchterlony, O.: *Acta path. et microbiol. scandinav.* **32:** 231. 1953.
12. Selye, H., J. B. Collip and D. L. Thomson: *Endocrinology* **17:** 494. 1933.
13. Geist, S. H., J. A. Gaines and G. C. Escher: *Endocrinology* **29:** 59. 1941.
14. Boyd, W. C.: *Fundamentals of Immunology,* Interscience Publishers, Inc., New York. 1947.
15. Rich, A. R.: Johns Hopkins Hospital Bulletin **98:** 120. 1956.
16. Hooker, C. W. and T. R. Forbes: *Endocrinology* **41:** 158. 1947.
17. Selye, H.: *Stress,* Acta, Inc., Montreal, Canada. 363. 1950.
18. Hollaender, A.: *Radiation Biology.* vol. 1, Part II. McGraw-Hill Book Co., Inc. 1954.
19. Furth, J.: *Cancer Res.* **13:** 477. 1953.

CHANGES IN THE PITUITARY GONADOTROPHS OF THE VITAMIN E-DEFICIENT MALE RAT

W. E. GRIESBACH, MURIEL E. BELL AND MURIEL LIVINGSTON

Endocrinology and Nutrition Research Departments of the Medical Research Council of New Zealand, Medical School, Dunedin, New Zealand

PURVES and Griesbach (1) have reported that in the rat pituitary two different types of gonadotrophic basophils could be found. One of these types was situated at the periphery of the gland in the proximity of the large portal vessels. The other type, less conspicuous in the normal animal, is located preferentially in the central regions of the anterior lobe. These two types of cells were named "peripheral" and "central" gonadotrophs and it was suggested that the peripheral type might be responsible for producing follicle stimulating hormone (FSH cell) while the central gonadotrophs might produce the luteinizing hormone (LH cell). Further evidence in support of this view has been obtained by Purves and Griesbach (2, 3). The existence of two types of gonadotrophs was also convincingly demonstrated by the electron microscopic studies of Farquhar and Rinehart (4).

Further information on the specific function of the two cell types has been obtained by a study of the pituitaries of vitamin E-deficient male rats. Since the work of Mason it is well known that vitamin E deficiency, like experimental cryptorchidism and x-ray irradiation of the testes, destroys the germinal epithelium of the tubules while the interstitial cells and the accessory sex glands remain intact (5, 6). Von Wagenen (7) and, in a classical paper, Nelson (8) have reported that pituitaries of male rats fed a vitamin E-deficient diet for at least six months showed mild but unequivocal castration changes.

The fact that vitamin E deficiency produces only a partial castration effect, suggested the possibility that the effect on the pituitary cells might likewise be a partial one. We have therefore followed the time course of the pituitary cell changes and attempted to correlate them with the changes in the gonads.

MATERIALS AND METHODS

Diet

The peameal-casein (PMC) diet used was as follows:—

Peameal (Pisum arvense)	50%
Casein	10%
Cooked starch	34%
Salt mixture (McCollum's)	4%
Cod liver oil	2%
	100%

Received October 12, 1956.

Evidence for vitamin E deficiency in the diet is provided by the males described in this paper. In the females abortion or resorption of foetuses occurred frequently. When young were delivered at term they were puny or nonviable. Further evidence of vitamin E deficiency was found in the *in vitro* response of the animals' washed erythrocytes to the effect of dialuric acid (9).

Experimental Animals:

The rats used were from a Wistar strain. They were weaned at 4 weeks and were then fed the PMC diet.

A group consisting of 4 males and 4 females, united at the age of 8 weeks in mixed pairs by the parabiosis technique, were fed for 4 weeks a stock diet after being joined, then transferred to the E-deficient diet. They remained in excellent condition.

Histological Methods

The pituitaries were examined as described by Purves and Griesbach (10). The periodic acid-Schiff method (PAS) was used for staining. The testes contained much plasma-like fluid which was allowed to escape by piercing the capsule before fixation in Bouin's fluid. They were weighed, together with the accessories, after 24 hrs of fixation. The sections were stained with hematoxylin and eosin acid-Schiff or periodic (PAS).

Assay for Pituitary Gonadotrophins

Pituitaries were assayed for gonadotrophic hormone content by injection of the homogenized tissue into 21-day-old female rats. The recipients got the equivalent of one pituitary divided into six injections over three days. For comparison, litter mate recipients were injected with pituitaries from normal males of the same age. In a number of experiments pituitaries from males that had been castrated at the beginning of the experiment were likewise assayed.

RESULTS

Animals were killed at varying intervals after the inception of the feeding of the PMC diet and are described below.

Effects on Pituitaries

4 Weeks. The testes of two rats, examined after 4 weeks on the peameal (PMC) diet, were of normal size and showed only a small number of tubules without spermatozoa corresponding to stage 1 of Mason (6). No definite castration changes were seen in the pituitaries.

5 Weeks. A group of 8 rats which had been on the PMC diet for 5 weeks had normal sized testes containing a greater number of damaged tubules (st. 1–2). Three pituitaries were normal but five showed an increased number of FSH cells, many of which were enlarged and partially degranulated. Where such degranulation had occurred, a rim of coarse glycoprotein granules round the edge of the cells formed a stronger staining ring. In some of these pituitaries a few LH cells were found to be enlarged and richer in glycoprotein than normal while in others, where the FSH cells were increased in number, the LH cells were undeveloped and resembled those in the immature animal (11) (Figs. 1 and 2).

7–9 Weeks. Between 7 and 9 weeks, the testicular degeneration in all rats

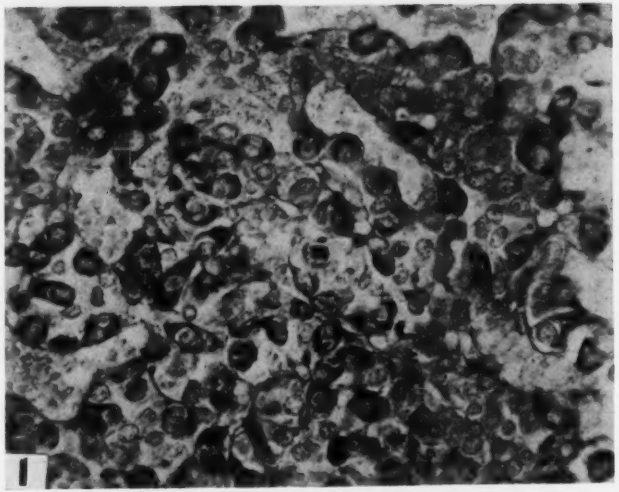
on PMC reached stage 2. There was as yet no significant change in testicular weight. In all pituitaries the FSH cells had further increased in number and were enlarged to a greater extent than the LH cells which were present in a few small clusters.

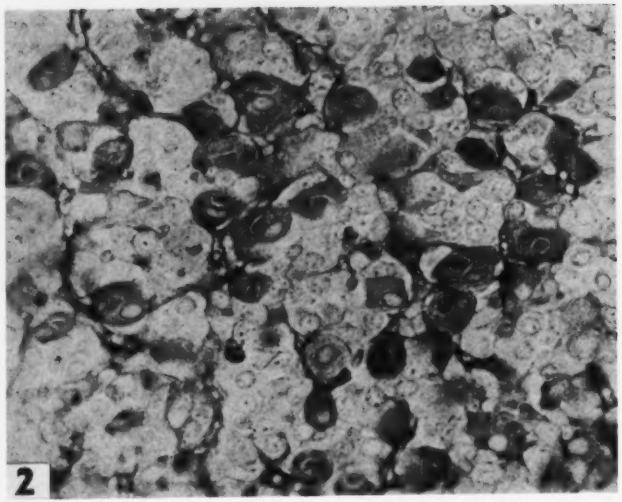
14–18 Weeks. Two more groups of 4 rats were examined at 14 and 18 weeks. The destruction of the germinal epithelium had then reached stages 2 and 3 respectively and a marked loss of testicular weight had taken place in the rats of the older Group 5 (Table 1). The cell changes in the pituitaries of Groups 4 and 5 did not reflect, to any marked degree, the spectacular fall in testicular weights and will be reported here together. The pituitaries contained numerous large FSH cells, some of which showed hyaline castration vesicles. The glycoprotein content of these cells was low and the negative image of the Golgi body was enlarged to a degree which is not commonly seen in the FSH cells of total castration. This was considered to indicate rapid hormone secretion (Figs. 3 and 4). The LH cells were not generally increased in numbers throughout the gland but were present in isolated clusters. Some of these cells had lost their characteristic large Golgi image and others were of the signet ring type with advanced hyalinization.

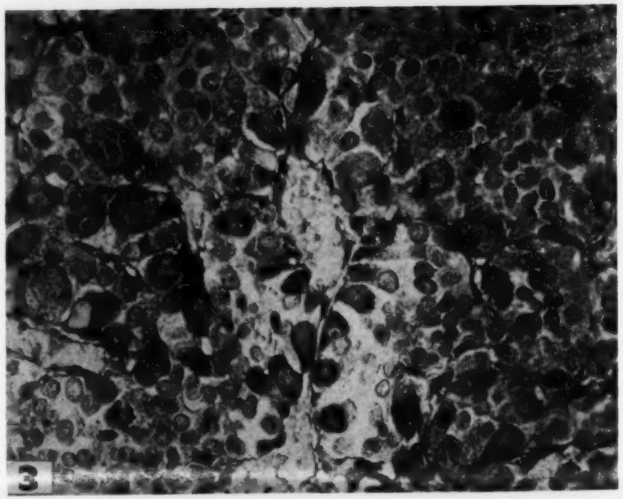
22 Weeks. In the 8 animals examined (only 4 are reported in Table 1) the weight of the testes had decreased still further and 6 had reached stage 5 of destruction while two animals had reached only stage 3. The severely degenerated testes contained plasma-like fluid. The weight of the accessories had increased further. The pituitary FSH cells once more far outnumbered the LH cells. All FSH cells were greatly enlarged irrespective of whether or not they contained vesicles. The glycoprotein content of many cells did not appear to be increased (Figs. 5 an 6). In some pituitaries the LH cells showed signs of regression and were difficult to identify, while in other cases they were quite active and similar to the type seen one month after castration (Figs. 7 and 8).

24–61 Weeks. Sixty glands were examined histologically. As was to be expected from the communications of Nelson (8) and Koneff (12), castration changes were found which were typical in character, but did not extend over the whole anterior lobe. Signet ring cells were frequently seen, favoring sites near the larger blood vessels. It was not always possible to recognize the specific cell type with certainty because vesiculation and other regressive changes interfered with the definition of the glycoprotein granules. Nevertheless the impression was obtained that in most pituitaries the FSH cells were still predominant in numbers and were actively secreting cells while some of the LH cells were poorly stained and showed signs of regression. It should be pointed out, however, that after about 6 months many active LH cells could be found.

After 39 weeks on the PMC diet two rats were castrated. They were killed 22 weeks later and their pituitaries showed the full development and





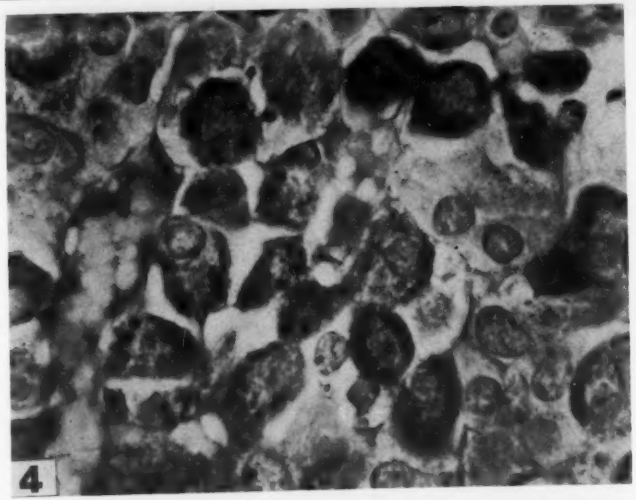


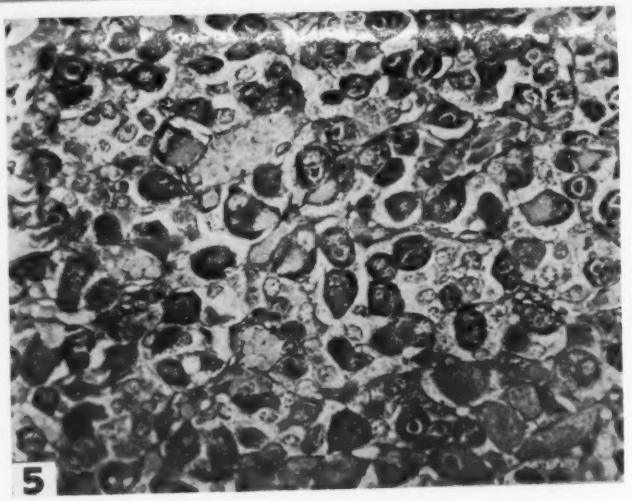
FIGS. 1–6 are photomicrographs of sections of male rat pituitaries stained with PAS and hematoxylin.

FIG. 1. FSH cells after 5 weeks on PMC diet. Increased number of perivascular cells showing dense glycoprotein granules. ×400.

FIG. 2. LH cells of same section, slightly increased in numbers and size. Golgi rings visible, surrounding glycoprotein granules of darker shade than the outer cytoplasmic granules. ×400.

FIG. 3. FSH cells after 14 weeks. Enlargement and degranulation is visible in most cells containing glycoprotein. ×400.





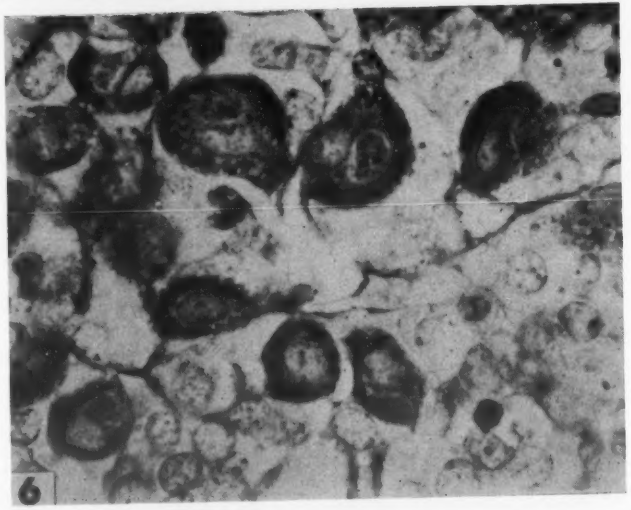
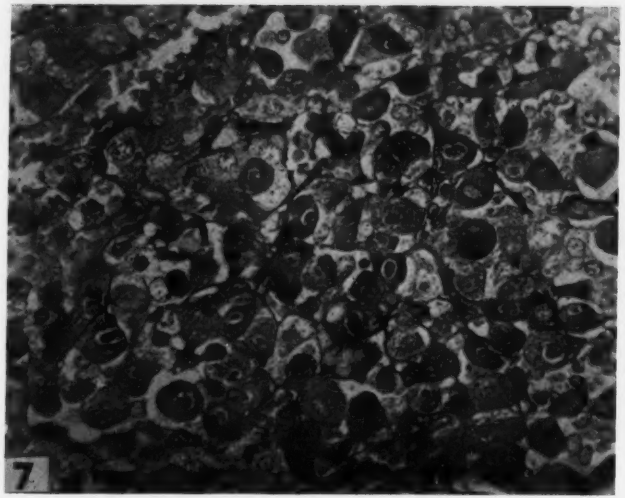
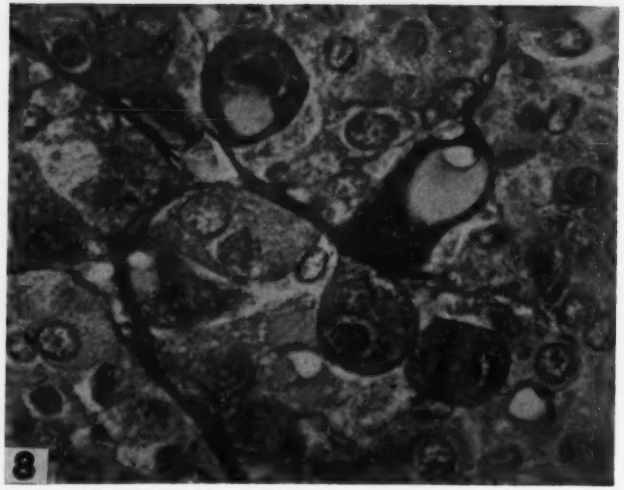


FIG. 4. Same section as Fig. 3, showing advanced degranulation in most FSH cells. ×900.

FIG. 5. FSH cells after 22 weeks. Most cells are enlarged, the granules are situated along the cell margins. Some Golgi rings and a few vesicles are visible. ×400.

FIG. 6. Same section as Fig. 5, showing clearly the cell sizes and distribution of the dense granules. ×900.





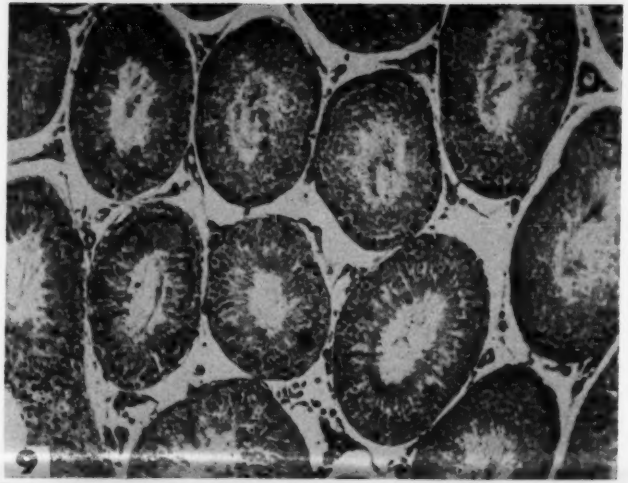


FIG. 7. LH cells in same section as Fig. 5. Large cluster of uniformly shaped "pale" cells with prominent Golgi rings. PAS and hematoxylin. ×400.

FIG. 8. Same section as Fig. 7, showing LH cells, two of them "signet ring" cells, in greater detail. PAS and hematoxylin. ×900.

FIG. 9. Section of rat testis after 14 weeks on PMC diet. Degeneration of tubuli at stage 1–2. Note small size of interstitial cells. H&E. ×90.

FIG. 10. Section of testis after 18 weeks on PMC. Some tubuli show the characteristic multinuclear giant cells of stage 3. H&E. ×90.

FIG. 11. Section of testis of rat 22 weeks on PMC. Stage 5 of tubular degeneration. Interstitial cells slightly hyperplastic. H&E. ×90.

FIG. 12. Sections of prostate of rat: (A) after 4 weeks; (B) after 22 weeks; and (C) after 61 weeks of PMC diet. Height of epithelial cells and size of Golgi bodies remain unchanged. H&E. ×400.

expansion of LH castration cells and were therefore in striking contrast to intact rats which had been deficient in vitamin E for the same interval of time.

Effects on Gonads

The effects of the PMC diet on the weights of the testes and the combined accessories of rats after 4–22 weeks are shown in Table 1A.

It was apparent that the relative weights of the testes tended to fall as the time on the diet increased, but the response was slightly irregular, as has also been noted by Nelson (8). A decided and permanent fall in the absolute and relative testicular weights, however, was seen to occur between 14 and 18 weeks, when the values dropped to less than one half of

TABLE 1A. WEIGHTS OF SEX ORGANS OF MALE RATS ON PMC DIET

Group No.	No. of rats	Wt. of rats gm. (av.)	Weeks on PMC diet	Wt. of testes		Wt. of accessories*		Stage of tubular degeneration
				Gm. (av.)	% b.w.	Gm. (av.)	% b.w.	
1	2	130	4	2.146	1.65	0.183	0.14	1
2	8	165	5	2.315	1.40	0.424	0.26	1–2
3	8	175	7–9	2.075	1.19	0.374	0.21	2
4	4	202	14	2.665	1.32	1.375	0.68	2
5	4	265	18	1.139	0.43	1.575	0.59	3
6	4	285	22	1.466	0.50	1.659	0.58	(3)–5
7	2	321	61	1.046	0.32	2.286	0.71	5
8	2†	315	61	—	—	0.194	0.06	—

* Accessories = total prostate plus seminal vesicles plus coagulating glands.
† Also castrated for 22 weeks.

those found in the younger animals. The weights of the accessories, on the other hand, showed an even larger rise between 9 and 14 weeks (i.e. between 13 and 18 weeks-of-age). The accessories continued to grow and reached unusually high weights, as described before by Drummond *et al.* (13) in vitamin E-deficient rats (Figs. 13 and 14). The accessories were affected in the usual manner by subsequent castration (group 8).

The histological changes in the testes (Figs. 9, 10 and 11) were similar to those described in the classical paper of Mason (6) and will not be discussed here in detail. After the destruction of the spermatopoietic apparatus, Sertoli cell nuclei were seen near the tubular membrane in a number of tubuli, while they were absent in others. The Leydig cells were apparently normal during the first months of PMC diet but they were increased in number and size after 22 weeks, at the time when the tubular damage had advanced to a high degree (Fig. 11). The intertubular spaces at this time were filled with fluid. After 61 weeks the size of the Leydig cells had not further increased but these cells were still secreting androgen, as shown by the active state of the prostate epithelium (Fig. 12). At a later stage (i.e. during the second year of vitamin E deficiency) we did find a number of rats in which degeneration of the accessories had followed the tubular

degeneration in the testes. This fact seems to prove a degeneration of the interstitial cells at a late stage of vitamin E deficiency. We have not seen nodular hyperplasia of Leydig cells in any of the PMC animals.

Bio-assay of Gonadotrophin. The gonadotrophic hormone content of the pituitaries of the vitamin E-deficient rats was similar to that of the normal male and was lower than that found in the pituitaries of castrated rats. Pituitaries of PMC rats are compared with those of normal rats in Table 1B. The majority of the recipients' ovaries contained mostly follicles with only a few corpora lutea. The pituitaries of 3 rats kept for 48–52 weeks on

TABLE 1B. GONADOTROPHIN ASSAYS OF PITUITARIES OF GROUPS 2–6 AND OF NORMAL MALES

	PMC diet	Normal males
No. of assay rats (21-day old females)	12	12
Mean weight of test ovaries	37.98 mg.	34.00 mg.
S.E.M.	±4.70	±4.45
S.E.D. 2.045, $t = 1.946$, $P > 0.05$		

the PMC diet, however, produced strong luteinization in quite small test ovaries of which one pair belonged to a hypophysectomized rat.

Parabiosis. A gonadotrophic assay by the method of parabiotic junction was performed on four couples of mixed sex. They were kept on PMC diet for 24–36 weeks after they had been joined for 4 weeks. The results of this experiment were inconclusive. The effect of vitamin E deficiency was not apparent in the testes of one male (I) after 24 weeks, and was retarded (stage 3) in another male (II) after 36 weeks. The testes of the remaining two males (III and IV) had reached stage 5 at 32 and 36 weeks. The female partners of the last two males (III and IV) were in continuous oestrus and had large uteri, filled with turbid fluid (pyometra). The ovaries contained only follicles and the mammary glands were stimulated. The gonads of the female joined to the unaffected male (I) were normal, while the female of pair (II) had large ovaries consisting of old corpora lutea only and the vagina was strongly mucified.

DISCUSSION

Nelson in 1933 (8) described the pituitary structure of rats on a diet deficient in vitamin E. He showed that in the male hypophysis, after 5 to 9 months, definite castration changes occur, but that these changes are not as great as those seen in the pituitaries of castrated animals. Koneff (12) gave a detailed description of the cytological changes in the basophil cells in the anterior hypophysis of vitamin E-deficient male rats. He noted the marked increase in cell numbers and sizes, the formation of signet ring types, and the enlargement of the Golgi rings.

The results of Nelson, Koneff and others have been confirmed in the present paper. One discrepancy between Nelson's (8) report and our own

observations concerns the first appearance of the "castration" changes in the pituitary. Nelson found that tubular damage in the testes occurred before the pituitary basophil reaction. His rats showed no appreciable or constant pituitary cell changes during the first 4 months, yet the tubular changes in the testes at that time had advanced up to stages 4–5. In contrast we found the pituitary to be a sensitive and early indicator of vitamin E-deficiency changes in the testes, reacting in some animals after 5 weeks of PMC feeding. The use of the PAS method may facilitate the recognition of early castration changes in the pituitary. This applies to the condition under discussion where early stages of the transformation of the normal FSH gonadotrophs are visualized with difficulty with conventional staining but are quite obvious with the use of PAS.

Our observations indicate that at about 5 weeks of PMC feeding a transformation of the pituitary FSH gonadotrophs takes place, strongly suggesting an increased secretion of FSH. It seems reasonable to assume that this transformation is due to the onset of tubular damage in the testes. Further increase in size and numbers of the FSH gonadotrophs parallels the progressive degeneration of the seminiferous apparatus up to 18 to 22 weeks when in most rats the spermatogenic epithelium of the testes is destroyed. At this stage the pituitaries resemble those of 15-day castrates (3).

It should be noted, however, that the LH cells are not completely unaffected. They increase in number and size, and form typical early and late castration type cells, with enlarged Golgi bodies and conspicuous nucleoli in large vesicular nuclei, as described by Koneff (12). The hyperplasia of the Leydig cells seen from 22 weeks onwards is almost certainly due to an increased production and release of LH from the activated LH gonadotrophs. A correlation between hyperplasia of the LH cells and hyperplasia of the Leydig cells in cryptorchid rats has been reported by Bielschowsky and Hall (14) and Bielschowsky (15).

The assays for pituitary gonadotrophic hormones up to 22 weeks of vitamin E deficiency, i.e., up to the destruction of the seminiferous epithelium, showed gonadotrophic potencies which were not higher than in normal males of the same age. Nelson (8) and Drummond *et al.* (13), who examined the pituitaries of rats which had been on vitamin E-deficient diet for 6 months or more, reported gonadotrophin values which lay between the normal and the castrate levels.

While our knowledge of the hormones produced by the testicle is far from complete, the action of the gonadotrophins on the testes is better known. There is much evidence for the assumption that the tubular apparatus is under the control of the follicle stimulating hormone while the interstitial cells depend for action on the presence of the pituitary luteinizing hormone (16, 17). After castration, the absence of both tubular and interstitial apparatus causes the rapid growth of first the FSH cells and

later of the LH cells (3). In vitamin E-deficiency states the degeneration of the tubular apparatus alone produces hyperplasia of the cells which Purves and Griesbach considered to be FSH cells. The increase in number and activity of the LH cells (which, however, remained at all stages less than that of the FSH cells and certainly far below the LH cell growth after castration) may account for the increase of the interstitial cells which was seen from 22 weeks onward (Fig. 11). This observation could explain the hypertrophy of the accessories described in this paper (Table 1). Drummond *et al.* (13) have described a similar hypertrophy in vitamin E-deficient rats (9–10 months). They were able to increase such elevated accessory weights even more by giving a preparation of pregnant mare serum which had a luteinizing action.

Purves and Griesbach suggested that the peripheral cell type might be responsible for FSH secretion while the central cell type might represent the site of LH secretion (2, 3). Since then Barrnett *et al.* (18) reported that the peripheral cell granules, in contrast to those of the central cell, could be easily dissolved in 2.5% trichloracetic acid. Pituitaries treated in such a way showed a predominantly luteinizing effect on bioassay. The authors therefore considered the peripheral, acid-soluble cell to be follicle stimulating. In disagreement with these findings is a recent report of Rennels *et al.* (19), who confirm the existence of two gonadotrophic basophils, but ascribe to them functional properties which are the reverse of those suggested in this paper.

SUMMARY

Feeding male rats a peameal-casein diet produces degenerative changes in the germinal epithelium of the testes within a short time, while the interstitial cells are maintained in a state of normality or of slight hyperplasia for over a year.

In response to the degeneration of the seminiferous epithelium the "peripheral" gonadotrophs of the pituitary increase considerably in number, size, and secretory activity while the "central" gonadotrophs show signs of stimulation to a minor degree only.

This condition of the gonadotrophs may be regarded as corresponding to the early follicle stimulating stage of pituitary basophil cell changes after castration.

These observations are considered to support the hypothesis that FSH is produced by the "peripheral" gonadotrophs.

REFERENCES

1. Purves, H. D. and W. E. Griesbach: *Proc. Univ. Otago Med. School* **30**: 27. 1952.
2. Purves, H. D. and W. E. Griesbach: *Endocrinology* **55**: 785. 1954.
3. Purves, H. D. and W. E. Griesbach: *Endocrinology* **56**: 374. 1955.
4. Farquhar, M. G. and J. F. Rinehart: *Endocrinology* **54**: 516. 1954.
5. Mason, K. E.: *J. exp. Zool.* **45**: 159. 1926.

6. Mason, K. E.: *Am. J. Anat.* **52:** 153. 1933.
7. von Wagenen, G.: *Anat. Rec.* **29:** 398. 1925.
8. Nelson, W. O.: *Anat. Rec.* **56:** 241. 1933.
9. György, P. and C. S. Rose: *Ann. New York Acad. Sc.* **52:** 231, 1949.
10. Purves, H. D. and W. E. Griesbach: *Endocrinology* **49:** 264. 1951.
11. Siperstein, E., C. W. Nichols, Jr., W. E. Griesbach and I. L. Chaikoff: *Anat. Rec.* **118:** 593. 1954.
12. Koneff, A. A.: *Anat. Rec.* **74:** 383. 1939.
13. Drummond, J. C., R. L. Noble and M. D. Wright: *J. Endocrinol.* **1:** 275. 1939.
14. Bielschowsky, F. and W. H. Hall: *Proc. Univ. Otago Med. School.* **32:** 16. 1954.
15. Bielschowsky, F.: *Proc. Univ. Otago Med. School.* **33:** 17. 1955.
16. Evans, H. M. and M. E. Simpson: The Hormones II: 360, 385. Academic Press, New York. 1950.
17. Paschkis, K. E. and A. E. Rakoff: The Hormones III: 864. Academic Press, New York. 1955.
18. Barrnett, R. J., A. J. Ladmán and N. J. McAllaster: *J. Histochem. Cytochem.* **3:** 391. 1955.
19. Rennels, E. G., J. E. Hildebrand and J. C. Finerty: Am. Soc. Zoologists 1956 (Personal Communication).

PLASMA 17-HYDROXYCORTICOSTEROID LEVELS AND CONDITIONED BEHAVIOR IN THE RHESUS MONKEY

JOHN W. MASON, JOSEPH V. BRADY AND MURRAY SIDMAN

Department of Neurophysiology and Experimental Psychology, Walter Reed Army Institute of Research, Walter Reed Army Medical Center, Washington, D.C.

ALTHOUGH the effects of physical factors on the pituitary-adrenocortical system have been extensively studied in the past two decades, the evaluation of the role of psychological factors in the regulation of ACTH secretion has been seriously hampered by the scarcity of techniques suitable for the systematic study of behavior in the individual experimental animal. Recent advances in animal conditioning methods within the field of experimental psychology, however, promise to provide a more refined approach to the investigation of such psychophysiological relationships. A relatively broad spectrum of behavioral patterns can now be operationally identified and experimentally manipulated. Specific aspects of the animal's emotional reactions can be selectively and quantitatively evaluated against the background of stable behavioral baselines (e.g. 1, 2, 3). The present report describes a series of experiments with Rhesus monkeys in which operant conditioning techniques (4, 5) have been applied to the study of relationships between behavior and pituitary-adrenocortical activity, using plasma 17-hydroxycorticosteroid (17-OH-CS) levels as an index of ACTH secretion.

METHODS

General Procedure. Nine adult Rhesus monkeys (*Macaca mulatta*) of both sexes, weighing between 3 to 5 kilograms, were longitudinally studied in these experiments. Plasma samples for analysis of 17-hydroxycorticosteroids were obtained before and after behavioral conditioning sessions of one or two hours duration. Three categories of behavioral responses were studied: lever pressing for food reward under several conditions, conditioned "anxiety" or "fear," and conditioned avoidance. Five monkeys were used in both the lever pressing for food reward and the conditioned "anxiety" experiments and in each animal hormone measurements were made in two or more sessions with each type of behavioral response. Four monkeys were used in the experiments on conditioned avoidance behavior with hormone measurements in three or more sessions with each animal. In all of the monkeys plasma 17-OH-CS response to a standard dose of ACTH was determined as a frame of reference for the interpretation of the changes observed in the same animals during conditioning sessions.

Physiological. Blood samples (5 to 7 ml.) were withdrawn into a heparinized syringe from the saphenous vein while the animals were briefly restrained on a foam rubber

Received November 10, 1956.

cushion. Samples were immediately centrifuged and the plasma separated and stored in the frozen state until analyzed. The concentration of free 17-hydroxycorticosteroids in each sample was determined by the Nelson-Samuels method (6, 7). Normal responsiveness of the adrenal cortex in each animal was established by measurement of the plasma 17-hydroxycorticosteroid response to a single intravenous injection of ACTH (16 mg./kg.). This dosage has been shown previously to produce a maximal rate of plasma 17-OH-CS rise of approximately 20–25 μg.%/hr. for at least two hours following injection (8). Experiments were carried out in all animals at regular intervals, in most instances about once a week. Hematocrit determinations were made periodically throughout the period of study.

Psychological. The apparatus employed in conditioning experiments consisted of a modified monkey cage, equipped with a hand lever operating a microswitch, and a series of devices for presenting to the animal small portions of solid or liquid food, mild electrical shock through a grid floor, and signals such as tones, clicking noises, or flashing lights. This cage was placed in a sound resistant room in such a way that the animal could be observed through a one-way vision mirror. Automatic control for the programming or scheduling of these stimuli in any desired sequence or combination was provided by a system of relays and timers. Level pressing responses were recorded cumulatively on a kymograph. This apparatus has been previously described in considerable detail (9).

1. *Lever Pressing Training for Food Reward.* Monkeys, deprived of food and liquid for 24 hours or more, were trained to press a lever for a few drops of sugared orange juice. The conditions under which this reward could be received were varied in different experiments as follows: each lever press produces a reward (continuous reinforcement); 20 lever presses are required for each reward (20:1 fixed ratio reinforcement); or lever pressing responses are rewarded at varying intervals ranging between 10 and 120 seconds, averaging one every 60 seconds (variable interval reinforcement). Each of these conditions is associated with characteristic rates and temporal patterns of lever pressing (4, 5). An example of a typical cumulative record of lever pressing for food on a variable interval schedule, showing about 900 lever responses in an hour, is presented in Figure 1.

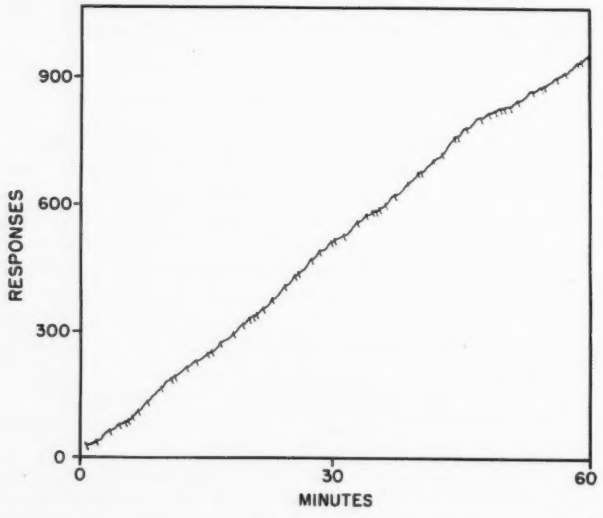


FIG. 1. Cumulative record of lever pressing on a variable interval schedule of reinforcement. The small vertical marks each indicate a food reward.

In general, this type of conditioned behavior has proven extremely useful as a background against which the effects of superimposed emotional disturbances can be measured. Ordinarily, the monkey will lever press at a relatively stable rate for many hours, but if a stimulus evoking a strong emotional response is superimposed at any point, this ongoing lever pressing activity may be immediately disrupted. A permanent record of such disruption of a stable pattern of lever pressing activity is obtained from the kymograph tracing.

2. *Conditioned "Anxiety"*[1] *or "Fear."* This conditioning situation is a striking example of how the ongoing behavior of lever pressing for food reward can be used as a background against which an emotional response may be measured. The response is established by superimposing upon lever pressing behavior the presentation of a steady clicking noise that terminates contiguously with an electric shock to the feet at the end of a 5-minute period. After only a few such clicker-shock pairings, the "anxiety" response appears as a disruption of the stable lever pressing pattern, accompanied by alternating crouching and intense locomotor activity, trembling, piloerection and frequently urination and defecation (10). Once established, this response to the clicker may be maintained even when the shock is only occasionally applied, and persists for some time after the shock is eliminated completely.

Blood samples were drawn under two different conditions during one-hour experimental sessions after establishment of the "fear" response. In the first, the clicker was sounded continuously for 45 minutes, and in the second, the one-hour session consisted of six 5-minute clicker presentations each separated by 5-minute periods of no clicker. No shocks were applied during these sessions. A cumulative record of the latter type of experiment, showing alternating periods of lever pressing when there is no clicker and suppression of lever pressing during the clicker, is presented in Figure 2. In all experiments, blood samples were drawn before, immediately after, and one hour after the termination of the session. Control procedures included measurement of plasma 17-OH-CS levels during sessions with the clicker alone before conditioning, and sessions with lever pressing alone after conditioning.

3. *Conditioned Avoidance.* In this related, but somewhat different, conditioning situation, monkeys were trained to press the lever to avoid an electric shock to the feet. The shock is delivered each time 20 seconds elapses without a lever pressing response. Every time the animal presses the lever, the shock is postponed for 20 seconds (11). Monkeys learned this response readily, stabilized at a high lever pressing rate, and not uncommonly performed without receiving a single shock during the two-hour sessions. A typical cumulative record of an avoidance session is shown in Figure 3. In addition to the high lever response rate these animals appeared very alert and were not easily distracted from the lever pressing task. Blood samples were drawn before and immediately after the two-hour sessions.

RESULTS

Lever Pressing for Food Reinforcement. Table 1 summarizes the findings in several groups of related experiments in which animals were lever pressing for food under various schedules of reinforcement. The "0" samples

[1] The term "anxiety" has been used in this report with certain reservations. We wish only to call attention to the fact that the response in the animal resembles in a number of respects the clinical state often described by this term. Little is to be gained by pressing this resemblance too seriously, particularly in view of the lack of agreement about what the term denotes clinically. At present it is probably advisable to adhere to an operational view of these responses in the experimental animal in terms of the conditions which produce them and of the measurable changes in behavior.

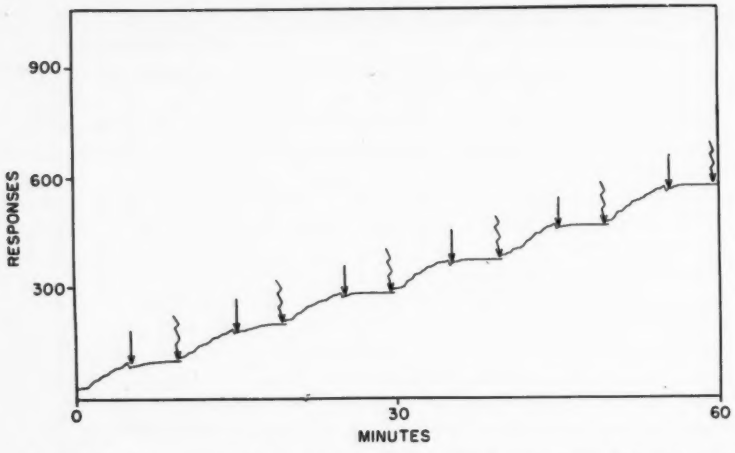


FIG. 2. Cumulative record of lever pressing with superimposed conditioned "anxiety" response. The straight arrows indicate the onset, and the jagged arrows the termination, of each five-minute clicker period. The pattern of lever pressing between clicker periods and suppression of lever pressing during clicker periods is evident.

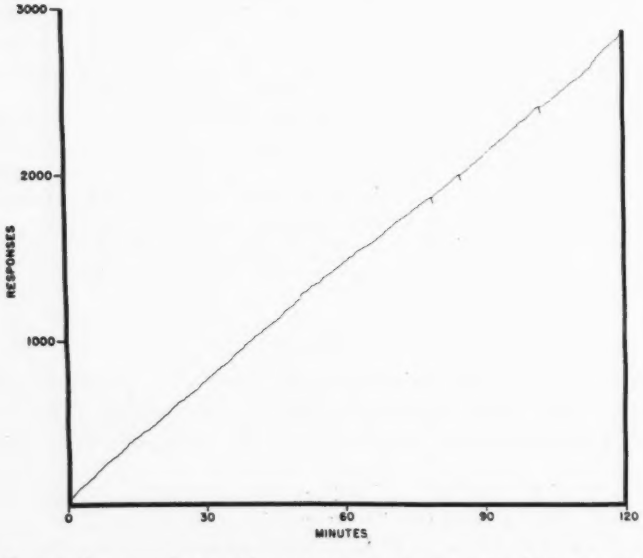


FIG. 3. Cumulative record of avoidance lever pressing, showing high rate of about 1500 responses per hour. The three small vertical marks indicate shocks occurring when 20 seconds elapsed between lever responses.

TABLE 1. PLASMA 17-HYDROXYCORTICOSTEROID LEVELS DURING CONDITIONING SESSIONS WITH VARIATION OF FOOD REWARD SCHEDULE*

Monkey No.	ACTH 16 mg./kg.		1st time in exp. cage		Lever pressing (cont. reinf.)		Lever pressing (20:1)		Lever pressing (20:1→100:1)	
	0	2 hrs.	0	2 hrs.	0	2 hrs.	0	2 hrs.	0	2 hrs.
1	20	68	40	63	51	42	35	42	43	34
2	41	79	56	67	64	47	54	34	50	42
3	29	61	20	47	45	41	38	26	74	45
Mean	30	69	39	59	53	43	42	34	56	40
Change during Session	+39		+20		−10		−8		−16	

* All values expressed as micrograms per 100 ml. of plasma. In instances where observations were made in multiple sessions on a single animal, mean values are used.

were taken immediately before the session and the "2 hrs." samples 2 hours after the beginning of a one hour session. Except for the first time each animal was placed in the experimental cage, no significant changes in plasma 17-hydroxycorticosteroid levels were associated with any of these lever pressing sessions, as compared with the responses obtained following administration of a large dose of ACTH (16 mg./kg.) by intravenous injection. This dose has previously been shown to elicit a maximal rate of plasma 17-hydroxycorticosteroid rise (20–25 μg.%/hr.) for at least two hours in the monkey (8). Zero time in these experiments was about 9 a.m. and the slight drops in level during the experiments are consistent with the normal diurnal variation in the monkey. In these early experiments the 9 a.m. baseline levels varied over a wider range than we have found in subsequent groups of monkeys. A number of values were above 50 μg.%, which we have come to regard as the upper limit of normal for this time of day. Although it was not possible systematically to isolate the factor(s) responsible for this widened range of baseline values, the occurrence of high values dropped markedly when the frequency of experiments was reduced from three to one or two experiments per week.

In none of the lever pressing for food sessions was there evidence of emotional disturbance except, perhaps, for a very transient anger-like response in the animals that were suddenly shifted from a 20:1 to a 100:1 fixed ratio schedule. This was characterized by pounding the lever at an increased rate and beating the walls of the cage, although the reaction in the monkeys appeared less intense than has been observed in other experiments. Additional samples drawn in these experiments 1 hour after the beginning of the session showed no elevation of corticosteroid levels.

Conditioned "Anxiety" or "Fear." Table 2 presents the results of the first of two sets of conditioned "anxiety" experiments which differed in the schedule of clicker presentation. In both experiments animals were conditioned with alternating 5-minute periods of clicker-plus-terminal

shock and 5-minute periods of no clicker. In the experiments shown in Table 2, however, the clicker was presented continuously for 45 minutes during the session in which hormone measurements were made. Table 2 shows that, following conditioning, the 45-minute clicking noise produces a moderate rise in plasma corticosteroid levels of about 10 μg.%/hr., whereas prior to conditioning mean values do not differ significantly from normal in sessions with the same auditory stimulus. However, the rise in these conditioned "anxiety" sessions is only about 50% of the maximal response which was achieved with ACTH administration in the same animals.

A second set of experiments was carried out with alternating 5-minute

TABLE 2. PLASMA 17-HYDROXYCORTICOSTEROID RESPONSE DURING ONE HOUR CONDITIONED "ANXIETY" SESSIONS

Control Sessions*				Conditioned "Anxiety" Sessions†			
Monkey No.	Plasma 17-OH-CS levels (μg./100 ml.)			Monkey	Plasma 17-OH-CS levels (μg./100 ml.)		
	Before session	At end of session	1 hour after session		Before session	At end of session	1 hour after session
1	34	37	30	1	42 33	50 41	44 38
2	60	42	42	2	50 39	66 48	53 36
3	21	28	20	3	40	54	—
Mean	38	36	31	Mean	41	52	43

* Control Sessions. With continuous clicker (45 min.) alone before conditioning.
† Conditioned "Anxiety" Sessions. With continuous clicker (45 min.) alone after conditioning.

periods of clicker and no clicker (but no shock), exactly as illustrated in Figure 2. Under these conditions, a maximal rate of plasma 17-hydroxycorticosteroid rise (20 μg.%/hr.) was observed, as shown in Table 3. Control sessions in this instance consisted of lever pressing sessions without the clicker, but after "anxiety" conditioning. In preliminary experiments it was found that, when such sessions followed series of "anxiety" sessions, corticosteroid elevations were sometimes observed, presumably related to a generalized response to the total situation. Such responses, however, can be readily adapted out by increasing the frequency of such control sessions without the clicker. The results in Table 3 show that under these conditions little, if any, change in corticosteroid level occurs in control sessions. Figure 4 shows the striking similarity between the plasma corticosteroid response during the "anxiety" sessions and that following the administration of a large ACTH dose in these animals. It also shows that the stimulation of the pituitary-adrenal cortical system ceases shortly after the session

TABLE 3. PLASMA 17-HYDROXYCORTICOSTEROID RESPONSE DURING ONE HOUR SESSIONS AFTER "ANXIETY" CONDITIONING

Control Sessions*				Conditioned "Anxiety" Sessions†			
Monkey No.	Plasma 17-OH-CS levels (μg./100 ml.)			Monkey No.	Plasma 17-OH-CS levels (μg./100 ml.)		
	Before session	At end of session	1 hour after session		Before session	At end of session	1 hour after session
6	47	54	36	6	35	56	—
	50	56	60		42	59	52
					55	85	63
7	46	39	38	7	61	80	—
	38	38	29		36	54	21
					48	63	52
Mean	45	47	41	Mean	46	66	47

* Control Sessions. Without clicker but after conditioning is established.
† Conditioned "Anxiety" Sessions. Sessions with alternating 5 minute periods of clicker and no clicker after conditioning is established.

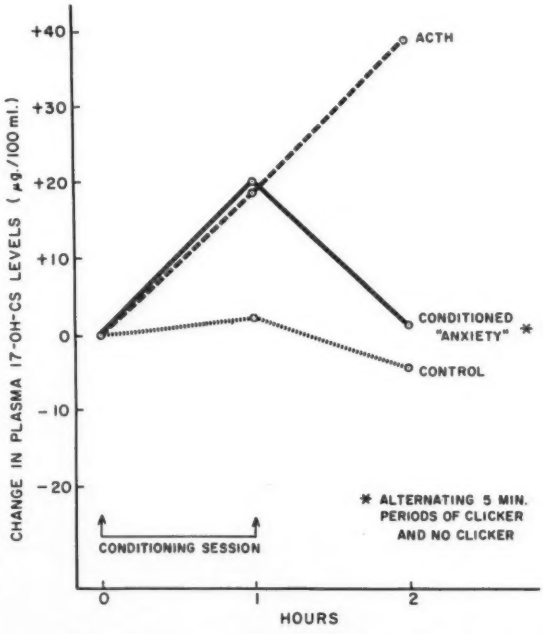


FIG. 4. Plasma 17-OH-CS response during conditioned "anxiety" sessions. The conditioned "anxiety" and control curves represent the mean values given in Table 3. In addition the similarity of the rate of rise in the "anxiety" sessions to that following I.V. ACTH (16 mg./kg.) injection is shown.

TABLE 4. MEAN PLASMA 17-HYDROXYCORTICOSTEROID RESPONSES DURING CONDITIONED AVOIDANCE SESSIONS

Monkey No.	Plasma 17-OH-CS levels (μg./100 ml.)		
	Before session	After session	Rise during session
4	19	55	36
5	41	78	37
12	20	50	30
25	31	68	37
Mean	28	63	35

Values shown represent mean of three or more experiments in each animal.

since one hour later values have returned almost to normal. It should be emphasized that the shock was never administered during experiments in which hormone measurements were made.

Conditioned Avoidance. Plasma 17-hydroxycorticosteroid levels invariably were markedly elevated after the two-hour avoidance sessions. Table 4 presents mean responses representing three or more experiments on each

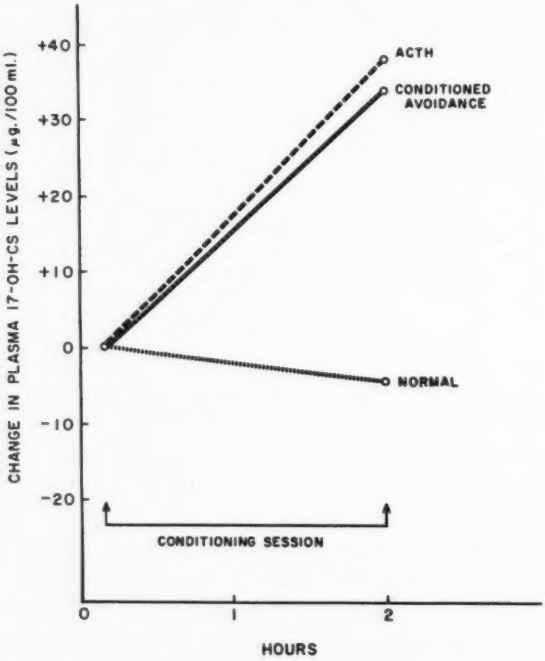


FIG. 5. Plasma 17-OH-CS response in monkeys during conditioned avoidance sessions. Avoidance curve represents mean values given in Table 4. In addition, a mean normal curve obtained from the same animals while in the home cage is shown, and the rate of rise during avoidance sessions is compared with that following the I.V. injection of ACTH (16 mg./kg.).

TABLE 5. PLASMA 17-HYDROXYCORTICOSTEROID RESPONSE DURING TWO HOUR CONDITIONED AVOIDANCE SESSIONS

Session No.	Monkey No. 25			Monkey No. 12		
	Plasma 17-OH-CS levels (μg./100 ml.)			Plasma 17-OH-CS levels (μg./100 ml.)		
	Before session	After session	Rise during session	Before session	After session	Rise during session
1	39	94	55	21	52	31
2	32	69	37	41	54	13
3	32	58	26	23	61	38
4	37	51	14	19	42	23
5	30	71	41	17	38	21
6	28	61	36	22	52	30
7	30	60	30	<2	35	34
10	32	80	48	19	65	46
15	15	69	54			
Mean	31	68	37	20	50	30

of four monkeys. The mean rate of rise is about 18 μg.%/hr. Figure 5 shows the similarity of this response to that following ACTH injection, as compared with the normal diurnal pattern for these animals at the same time of day while in the home cage. Table 5 shows the persistence of the corticosteroid rise in two animals followed with hormone measurements for seven consecutive weekly avoidance sessions and again on the tenth session. In every session significant corticosteroid elevations occurred, ranging from 13 to 55 μg.% during the two hour period. Figure 6 illustrates the apparently cyclic nature of the adrenal cortical response in a monkey fol-

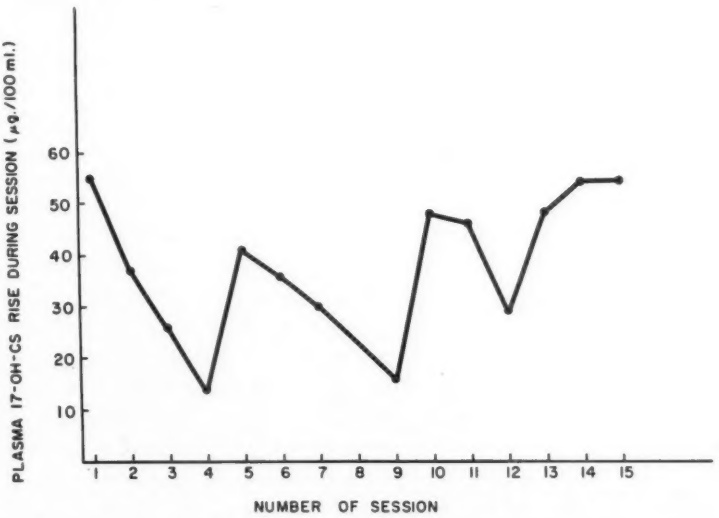


FIG. 6. Plasma 17-OH-CS responses of a monkey in fourteen consecutive avoidance sessions, showing an apparent cyclic pattern of response.

lowed for 15 consecutive weekly sessions. Monkeys proved to be quite adept in the avoidance situation and usually received less than four shocks, often none, in the 2 hour sessions. The corticosteroid response was always obtained, however, and appeared to be independent of the number of shocks.

DISCUSSION

In the experimental situations studied so far, the results clearly indicate that at least two types of conditioned behavior may be associated with marked elevations of plasma 17-OH-CS levels, namely, "anxiety" or "fear" and avoidance. Obviously, many additional types of behavioral responses remain to be similarly studied before some of the larger questions about the relationships between the pituitary-adrenocortical system and behavior can begin to be clarified. It is possible, however, to point out certain limited conclusions on the basis of the responses already studied since they can be differentiated both with regard to the conditions which elicit them and the overt reactions which characterize them. Although there is the threat of shock in both situations, in the case of conditioned "anxiety," the animal has no control over the shock. In the conditioned avoidance on the other hand, the animal can prevent the shock indefinitely by periodically pressing a lever. Certainly, the animal in the avoidance situation does not appear as disturbed as the crouching, trembling animal responding to the "anxiety" stimulus. Despite these behavioral distinctions, however, the adrenal cortical response appears to be maximal in both situations. In addition, previous studies have shown that plasma 17-OH-CS elevations can also be associated with such "emotionally" disturbing situations as the first handling and venipuncture of a new monkey and the first transfer from the home cage to an experimental cage (12). One of the questions to be answered is whether the pituitary-adrenal cortical response is correlated with a relatively specific component of these various situations, or with a more undifferentiated type of "emotional state" upon which a variety of distinguishable responses may be superimposed. Clinical studies have not yet helped much to clarify this problem. Positive correlations have been reported in human subjects between emotional disturbance of the "anxiety" type and ACTH secretion, as judged by relatively indirect indices such as urinary 17-ketosteroid excretion, lymphocyte and eosinophil count (13–16). Some findings in concurrent studies in our laboratory on emotional states and plasma 17-OH-CS levels in human subjects suggest that increased pituitary-adrenal cortical activity may be associated with a rather broad range of emotional responses (17). Perhaps the further study of a broader spectrum of behavioral responses in the experimental animal will permit relationships of this type to be additionally clarified.

A finding of some practical interest was that the adrenal cortical response to the "anxiety" and avoidance situations can be dependably reproduced and does not appear to be modified or "adapted out" by repeti-

tion over a period of many months. This fact should be encouraging to the investigator in the psychophysiological area who is seeking reliable, standard tools for eliciting emotional responses in the experimental animal. One of the methodological problems in assessing relationships between emotional responses and a relatively slow-moving physiological response like that of the pituitary-adrenal cortical system concerns the control of the duration of the emotional response. With this system it is generally more satisfactory to deal with stimulus situations which can be maintained fairly evenly over a period of at least an hour or more, rather than with stimuli of short duration even though of high intensity. This must be taken into account in the evaluation of results obtained with transient emotional responses like that following the sudden withdrawal of food reward from the lever pressing animal, i.e., extinction, or that associated with abrupt increases in the fixed ratio schedule. Both the avoidance and the "anxiety" responses, however, can be readily maintained for many hours.

Another aspect of the relationship between emotional responses and ACTH release is whether the two effects result concomitantly from the same stimulus or whether one is sequential to the other. Our data at this point simply show that they occur together under the conditions of our experiments. There is some indication that when the behavioral response varies markedly in intensity, the hormone response tends to vary in the same direction. In a recent study on the effects of reserpine on the behavioral and adrenal cortical responses to a conditioned "anxiety" stimulus, it was found that there was striking correlation between the changes in the two responses (10). During periods of reserpine administration both were greatly diminished, while during periods without the drug both responses returned to control levels. The only difference noted was that the endocrine response to the emotional stimulus did not reappear as rapidly as the behavioral response after reserpine withdrawal. This question of whether the relationship is concomitant or sequential is a difficult one and will probably be clarified only by the convergence of findings from a variety of different experimental approaches.

SUMMARY

An approach to the study of the relationships between emotional behavior and the pituitary-adrenal cortical system, using operant conditioning techniques in the monkey, has been described. Plasma 17-hydroxycorticosteroid levels were determined before and after a variety of behavioral conditioning sessions. Animals lever pressing for a food reward on a continuous or on a 20:1 fixed ratio reinforcement schedule showed no elevations in plasma 17-OH-CS levels. In addition, abrupt shifting from a 20:1 to a 100:1 fixed ratio schedule during a lever pressing session was not found to be associated with evidence of an adrenal cortical response. In two situations, however, conditioned "anxiety" and avoidance, marked elevations

(about 20 μg.%/hr.) of plasma 17-OH-CS levels invariably appeared. This rate of rise is similar to that following the single intravenous injection of 16 mg./kg. of ACTH and appears to represent a maximal rate of rise in the monkey. It was also observed that monkeys showed a substantial plasma corticosteroid rise the first time that they were brought from the home cage to the experimental cage. On the second occasion this effect did not occur and was not observed subsequently. It is believed that the further study of conditioned behavioral responses may prove a fruitful approach to a better understanding of the qualitative and quantitative relationships between behavior and the adrenal cortex.

Acknowledgment

The authors wish to thank Mrs. Nancy Mertz for helping with the chemical analyses and Mr. Earl Turner for his technical assistance.

REFERENCES

1. Estes, W. K. and B. F. Skinner: *J. Exper. Psychol.* **29**: 390. 1941.
2. Brady, J. V. and H. F. Hunt: *J. Psychol.* **40**: 313. 1955.
3. Azrin, N.: *J. Psychol.* **42**: 3. 1956.
4. Skinner, B. F.: *The Behavior of Organisms.* New York: Appleton-Century. 1938.
5. Ferster, C. B. and B. F. Skinner: *Schedules of Reinforcement.* New York: Appleton-Century-Crofts, in press.
6. Nelson, D. H. and L. T. Samuels: *J. Clin. Endocrinol. and Metab.* **12**: 519. 1952.
7. Harwood, C. T. and J. W. Mason: *J. Clin. Endocrinol. and Metab.* **16**: 790. 1956.
8. Harwood, C. T. and J. W. Mason: *Endocrinology*, in press.
9. Conrad, D. G. and M. Sidman: *Psychol. Rep.* 1956, in press.
10. Mason, J. W. and J. V. Brady: *Science* **124**: 983. 1956.
11. Sidman, M.: *Science* **118**: 157. 1953.
12. Mason, J. W., C. T. Harwood and N. Rosenthal: In preparation.
13. Pincus, G. and H. Hoagland: *Am. J. Psychiat.* **106**: 641. 1950.
14. Hoagland, H., G. Pincus, F. Elmadjian, L. Romanoff, J. Hope, J. Balian, A. Berkeley, and J. Carlo: *Arch. Neur. & Psychiat.* **69**: 470. 1953.
15. Cleghorn, R. A. and B. F. Graham: *Am. J. Psychiat.* **106**: 668. 1950.
16. Thorn, G. W., D. Jenkins, and J. C. Laidlaw: Adrenal response to stress in man. Recent Progress in Hormone Research Vol. 8, p. 171, New York, The Academic Press, 1953.
17. Price, D. B., M. B. Thaler, and J. W. Mason: *Arch. Neur. & Psychiat.* (in press).

ADRENALINE AND NORADRENALINE IN THE ADRENAL MEDULLA DURING POSTNATAL DEVELOPMENT OF THE RAT[1,2]

OLAVI ERÄNKÖ AND LIISA RÄISÄNEN

Department of Anatomy, University of Helsinki, Siltavuorenpenger, Helsinki, Finland

THE adrenal glands of fetuses and newborn animals of many mammalian species have been reported to contain more noradrenaline in proportion to the total catechol amine content than adrenals of adult animals (10, 11, 13, and others). However, the content of these catechol amines has usually been expressed either in terms of percent of *adrenal* weight, or as micrograms per gland, without data on the medullary volume. Little is therefore known of age dependent differences in the *medullary* concentration of adrenaline and noradrenaline. Moreover, all previous studies on the development of the adrenal medulla have apparently been made without knowledge of the fact that there are two different types of adrenomedullary cells, of which one secretes adrenaline, the another noradrenaline (2, 3, 6, 7, 8, 9).

The present report deals with observations made using both chemical and histochemical methods for the study of adrenaline and noradrenaline in the adrenal medulla of rats of varying ages. The rat is a suitable animal for a study like this because its adrenal medulla is essentially formed first after birth and because the adrenals of adult rats have been demonstrated to contain two kinds of medullary cells for the secretion of adrenaline and noradrenaline, respectively (6, 8).

MATERIALS AND METHODS

Adrenals from altogether 66 albino rats of the Wistar strain were studied. The animals were of both sexes but no significant sexual differences were observed in the variables concerned in the present problem, except in the adult rats. The size of the adrenal cortex is larger in adult females than in males. Figures referring to adult rats in this paper are obtained from males.

The animals were killed without any previous treatment by decapitation with sharp scissors, and the adrenals were carefully removed and dissected free of the surrounding adipose tissue.

Histological and histochemical methods

Right adrenals were used for histochemical study. They were immersed either into a mixture containing 1 volume of concentrated formalin, stored over calcium carbonate,

Received December 3, 1956.

[1] Part of the study was completed at the Institute of Occupational Health, Helsinki.

[2] Supported in part by a grant from the Sigrid Jusélius foundation to the senior author (O.E.).

and 9 volumes of 3.5% potassium dichromate, or into a saturated solution of potassium iodate (9). Glands fixed in dichromate-formalin were weighed after fixation and cut in a complete series of sections for planimetric determination of the medullary volume (4). Adrenals immersed in the iodate solution were subsequently fixed in 4% formaldehyde and cut with a freezing microtome. Unstained sections from glands treated with either method were examined after mounting in glycerol. Some sections from each gland were stained with sudan red, some others with Harris hematoxylin and sudan red. Some glands were dehydrated and embedded in paraffin wax after fixation; sections from such glands were stained with hematoxylin and eosin or with Masson's trichrome method.

Chemical methods

Left adrenals were directly applied on the base line of the previously acid-washed filter paper (Whatman No. 1) which was subsequently used for chromatographic separation of adrenaline and noradrenaline. If the gland was small, diameter *c.* 2 mm. or less, it was smeared as such on the base line of the paper. Adrenals of adult rats were frozen on the tissue holder of a freezing microtome and a complete series of fresh sections was cut at 50μ with a cooled knife. All sections were transferred frozen on the chromatogram paper where they were allowed to thaw.

Adrenal tissue thus transferred on the filter paper was allowed to dry. It was then extracted by wetting the base line twice with N/10 hydrochloric acid, whereafter ascending chromatography was run overnight at 26° C, using 15 percent (w/w) of N/10 hydrochloric acid in freshly distilled phenol (1, 5, 12). The paper was dried, rinsed in several changes of analytical benzene and cut into vertical strips each corresponding to one whole adrenal. One strip of each paper was sprayed with buffered potassium ferricyanide and the catechol amines were located as fluorescent spots under ultraviolet light. The other strips of the same paper were then cut horizontally according to the sprayed strip so that one horizontal substrip contained all adrenaline and the other all noradrenaline. These strips were eluted with 0.4% monosodium ortho phosphate. Adrenaline and noradrenaline concentrations of the eluates were determined fluorometrically (5). Since the presence of cortical tissue interferes with the running speed of adrenaline and noradrenaline in the chromatogram paper, and since cortical ascorbic acid has an R_f value intermediate to those of adrenaline and noradrenaline, the sprayed control strips were always prepared using adrenals from animals who were littermates with those whose adrenals were applied onto the same paper for elution and fluorometric determination. In all instances the separation of adrenaline, noradrenaline and ascorbic acid was clear-cut and the R_f values stable in different parts of the base line. Therefore, the method employed is particularly reliable for accurate determination of the adrenaline: noradrenaline ratio.

OBSERVATIONS

Histological and histochemical

In the adrenals of the newborn rats examined no clearly delineated adrenal medulla was observed. However, spherical groups of prospective medullary cells were seen in the center of the gland. Sudan-positive cortical cells separated these cell groups from each other. The intensity of the chromaffin reaction in the medullary cells was weak but the yellow color of these cells could nevertheless be noticed without difficulty in unstained sections cut from glands fixed in the dichromate-formalin mixture.

In a week after birth a definite adrenal medulla was already formed, and the border between the medulla and the cortex was as a rule sharp. In

sudan-stained sections it was observed, however, that heavily lipid-laden cells were scattered all over the medulla, and these cells were particularly numerous at the cortico-medullary junction. Perhaps these lipid-rich cells were rests of cortical cells which had remained between the fusing groups of medullary cells during the formation of the medulla. Although some sudan-positive cell groups presumably of cortical origin were regularly seen even in the adrenal medulla of adult rats, the relative number of such cells in the clearly delineated medulla was insignificant after the age of about 10 days.

The intensity of the *chromaffin reaction* steadily increased with increasing age. Although the intensity of the reaction was only subjectively assessed in this study, it was quite clear that the color of the adrenal medulla was more intense in 7-day-old rats than in newborn ones. Rats aged 2 weeks were on the color basis easily discriminated from those 7 days or 3 weeks old. The color produced by the dichromate-formalin in the adrenal medulla of 3 weeks old animals was brown, not any more yellow, but the intensity of the brown color in the medulla of adult animals was without doubt still stronger.

A positive *iodate reaction* was observed in the adrenal medulla of rats 9 days old or older. The failure to see any reaction in the adrenals of younger rats is most likely due to low noradrenaline concentration (see below). The pattern of iodate-positive adrenomedullary cells, i.e. cells presumably producing and containing principally noradrenaline, was the same in young and in adult animals. The main bulk of the medulla was essentially iodate-negative but clearly delineated groups of cells scattered in the medulla were positive. The intensity of the yellow color was stronger in the adult rats. As far as could be judged by mere inspection, the proportional amount of the iodate-positive cells in the medulla remained roughly the same throughout the growth period.

Adrenal growth and catechol amine content

In Figure 1 animal and adrenal weights, volume of the adrenal medulla, and contents of adrenaline and noradrenaline per gland are shown as plotted against age. The scales on both the abscissa and the ordinate are logarithmic, which makes direct comparison of the age-induced changes in each variable convenient. Every point represents a figure obtained from a single animal.

It may be seen that the shape of all the curves is sigmoid, as can indeed be expected knowing that growth curves generally are of the logistic type. The slopes vary, however, and the curves have been arranged from top downwards according to increasing slope. Thus it is seen that, during the period covered, the proportional increase in the medullary volume is smaller than that in the adrenal weight, *i.e.*, the relative amount of cortical tissue increases with advancing age.

The slopes of the noradrenaline and adrenaline curves resemble closely

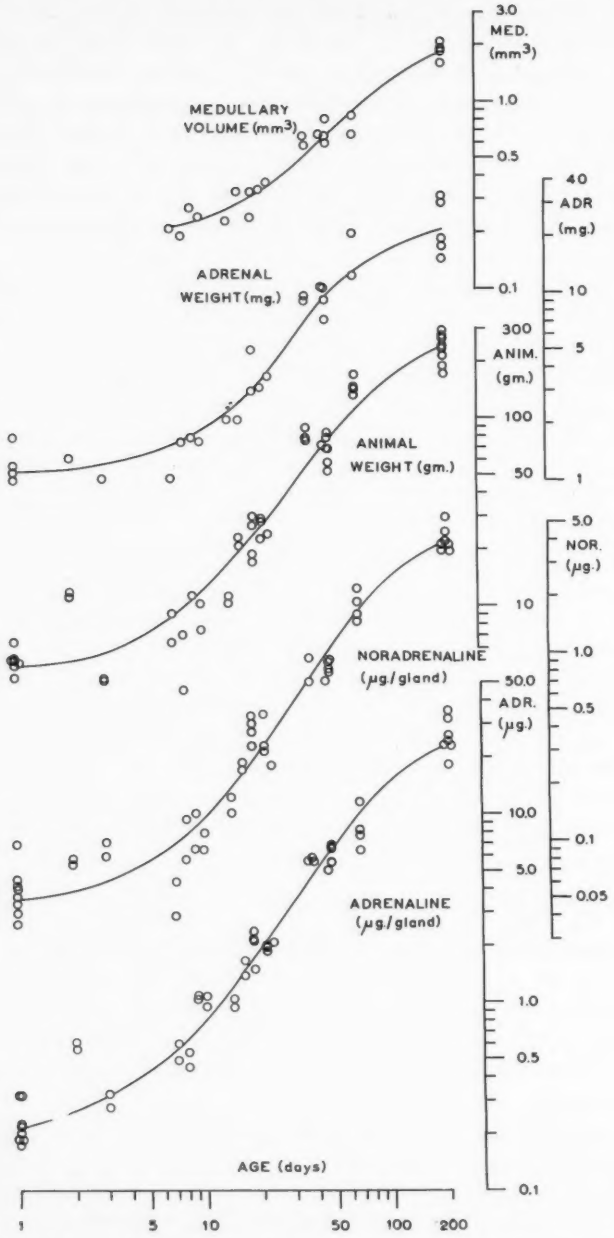


FIG. 1. Age-induced increases in the medullary volume, adrenal weight, animal weight as well as noradrenaline and adrenaline content of the adrenals. Each circle denotes an observation on one animal. The curves have been fitted to the data by eye. Both scales are logarithmic.

each other, which suggests that the relative increases in the amounts of these catechol amines are identical in the various stages of postnatal development. It seemed to be of particular interest to analyse more closely the apparent resemblance of these two curves. Since the curves were fitted by eye only, and since it proved cumbersome to find transformed variates which would straighten the curves, amounts of adrenaline and noradrenaline were plotted against animal weight, using again logarithmic transformation on both axes. The result is illustrated in Figure 2, in which the regression lines have been fitted to the data by calculation, using the method of least squares. It is easy to see that a good fit is obtained for both adrenaline and noradrenaline, and that the slopes of the two regression lines are closely similar. The slight difference in the slope is not statistically significant, as was also proved by statistical calculations.

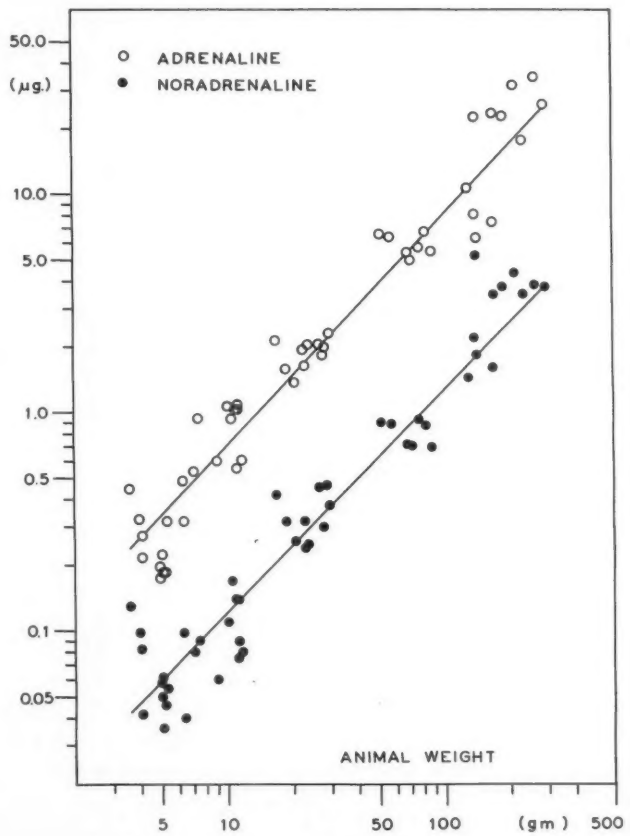


FIG. 2. Adrenaline and noradrenaline contents of adrenals plotted against animal weight. Note the closely similar slopes of the two lines, which have been fitted to the data by calculation. Both scales are logarithmic.

Some ratios are plotted against age in Figure 3, again using logarithmic coordinates. It is seen that, quite as could be expected from histochemical observations on the intensity of the chromaffin reaction, the medullary concentration of catechol amines notably increases with advancing age (upper rectangle). On the other hand, the relative volume of the medulla decreases in proportion to the weight of the whole adrenal (lower rectangle). Therefore, if the catechol amine content had been expressed in proportion to the weight of the whole adrenal, as has been usual in many previous papers, the increase in the medullary catechol concentration had in part been masked by the simultaneous shift in the ratio *size of the medulla*:*size of the cortex*.

It was not possible to obtain sufficiently accurate estimates of the medullary size for animals younger than a week, because no compact medulla

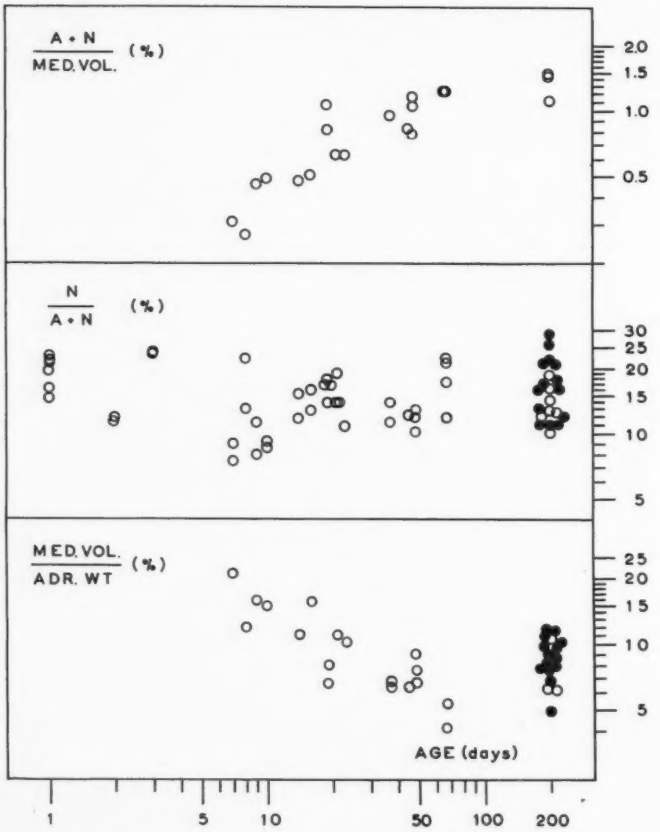


FIG. 3. *Concentration* of total catechol amines (top), noradrenaline percentage of total catechol amines (center), and relative volume of the medulla (bottom) in the adrenals as functions of age. All scales logarithmic. The full circles represent adult male values of previous experiments by the writers.

had yet been formed in the adrenals of these animals. It was nevertheless possible even in these cases to determine accurately the proportion of the noradrenaline content to the total content of catechol amines. This proportion was well within the normal adult range (center rectangle).

DISCUSSION

The present work confirms the earlier observations (10, 11, 13) that the amount of adrenal catechol amines steadily increases in the course of the development. The postnatal increase in the content of these amines is indeed very large, the amine content of the adrenal of an adult rat being about 100 times as large as that of a newborn rat.

The earlier observations which indicate that the ratio *noradrenaline-adrenaline* decreases with advancing age (10, 11, 13) are at variance with the findings of this work. Species differences are certainly in part responsible for the discrepancy. Thus, Shepherd and West (11) investigated adrenals of various mammals other than the rat, such as man, the cat, the dog, the rabbit, the guinea pig and the sheep, and although all other species showed a decrease in the relative noradrenaline content of the adrenals, no such decrease was seen in the sheep. Careful examination of Hökfelt's (10) data of catechols in the rat adrenal suggests that random fluctuations rather than a real change may be responsible for the apparent postnatal increase in the relative adrenaline content of his data. Individual variations in the relative amounts of adrenaline and noradrenaline were indeed considerable in his material and in the present work. However, the remarkable similarity of the slopes of the logarithmic adrenaline and noradrenaline curves (Fig. 2) demonstrate clearly that there is no real systematic age dependent change in the relative amounts of these two hormones in the adrenal medulla of the rat.

This finding is the more interesting as it was shown both by quantitative catechol amine determinations and histochemically that the adrenomedullary concentration of catechol amines increases almost five times in the course of the period from 10 to 200 days-of-age. At the same time, the growth of the adrenal cortex is more rapid than that of the medulla so that the relative volume of the medulla decreases with age. This speaks against the view that the degree of methylation of noradrenaline is related to the relative size of the cortical tissue (13).

Histochemical and histological examination showed that the formation of the adrenal medulla was still in progress after birth. It may furthermore be questioned whether the prospective adrenomedullary cells of the newborn rat, in which the chromaffin reaction indicated a low catechol amine concentration, really have any secretory activity at all. Hökfelt's figures indicate that the catechol amine content of the adrenals of rat fetuses three days before birth is only about one tenth of that in the adrenals of a newborn rat. The present writer's efforts to observe any chromaffin reaction in the adrenals of rat fetuses about three days before birth have remained

unsuccessful, which also proves the low concentration of catechol amines at this stage. Hökfelt's figures suggest a fairly high *noradrenaline:adrenaline* ratio in the fetal adrenals. This observation can be better understood, if the cells containing these amines are regarded as sympathicoblasts rather than secretory adrenomedullary cells, because cathecol amine concentration is known to be low and the *noradrenaline:adrenaline* ratio high in sympathetic ganglion cells.

Two observations made in the present study, i.e., firstly that the adrenaline and noradrenaline contents of the adrenal both increased at the same rate and, secondly, that the number of noradrenaline-secreting iodate-positive cells remained largely constant in proportion to the total number of adrenal cells, suggest that the chromaffin cells specialize from the very beginning of their function to the secretion of *either* adrenaline *or* noradrenaline, not of both, and that the rates of formation of both of these two types of cells are equal throughout the postnatal development.

SUMMARY

Adrenals of rats 0–200 days old were studied. Adrenal weight, volume of the adrenal medulla and the contents of adrenaline and noradrenaline were quantitatively determined. Histochemical chromaffin and iodate reactions as well as sudan staining were also employed.

Adrenomedullary cells of all animals showed a positive chromaffin reaction and contained both adrenaline and noradrenaline. The intensity of the chromaffin reaction and the medullary concentrations of adrenaline and noradrenaline increased with age. Although a hundredfold increase in the content of both catechol amines per gland was observed, the *noradrenaline:adrenaline* ratio remained essentially constant, as did the relative amount of noradrenaline-secreting cells in the medulla.

It is concluded that the adrenaline-secreting and the noradrenaline-secreting cells of the adrenal medulla differentiate early and that the rates of formation of both of these two types of cells are equal throughout the postnatal development.

REFERENCES

1. Crawford, T. B. B. and A. S. Outschoorn: *Brit. J. Pharmacol.* **6**: 8. 1951.
2. Eränkö, O.: *Acta physiol. Scand.* **25**: Supp. 89. 1951.
3. Eränkö, O.: *Acta anatom.* **16**: Supp. 17. 1952.
4. Eränkö, O.: *Ann. med. exper. biol. Fenniae* **32**: 377. 1954.
5. Eränkö, O.: *Ann. med. exper. biol. Fenniae* **32**: 392. 1954.
6. Eränkö, O.: *Endocrinology* **57**: 363. 1955.
7. Hillarp, N.-Å. and B. Hökfelt: *Acta physiol. Scand.* **30**: 55. 1953.
8. Hillarp, N.-Å. and B. Hökfelt: *Endocrinology* **55**: 255. 1954.
9. Hillarp, N.-Å. and B. Hökfelt: *J. Histochem. Cytochem.* **3**: 1. 1955.
10. Hökfelt, B.: *Acta physiol. Scand.* **25**: Supp. 92. 1951.
11. Shepherd, D. M. and G. B. West: *Brit. J. Pharmacol.* **6**: 665. 1951.
12. Vogt, M.: *Brit. J. Pharmacol.* **7**: 325. 1952.
13. West, G. B.: *Quart. Rev. Pharmacol.* **30**: 116. 1955.

HYPOKALEMIA IN ADRENALECTOMIZED DOGS DURING ACUTE DECOMPRESSION STRESS[1,2]

FREDERICK P. FERGUSON, DIETRICH C. SMITH AND JEANNE Q. BARRY

Department of Physiology, University of Maryland, School of Medicine, Baltimore, Maryland

IT HAS been previously reported from this laboratory that decompression of intact, unanesthetized dogs to a simulated altitude of 30,000 feet for 30 to 90 minutes consistently results in a decrease in the plasma potassium concentration (1). The present study was undertaken primarily to determine whether or not a similar response occurs in adrenalectomized dogs upon exposure to this altitude for a corresponding period of time. The desirability of such an investigation was indicated by recent reports that in the cat (2) and also in the rat (3) the characteristic plasma potassium response to altitudes of 28,000 to 30,000 feet is abolished by adrenalectomy and is therefore apparently mediated by the adrenal glands.

PROCEDURE AND METHODS

The study was carried out upon four bilaterally adrenalectomized female dogs weighing between 6 and 11 kg. Each animal was carefully trained to submit to the experimental procedure in order to obviate the use of an anesthetic. Following adrenalectomy, the animals were routinely maintained with injections of cortisone acetate (12.5 mg. per day, i.m.) or desoxycorticosterone acetate (DCA) (0.6–1.0 mg. per day, i.m.). Supplementary NaCl was administered while the animals were being maintained upon cortisone. Except for a few instances, in which moderate adrenal insufficiency was allowed to develop, injections were continued through the day preceding an experiment. Food was removed from the cage on the afternoon before an experiment and the dog was allowed free access to diluted evaporated milk until the following morning when the experiment was performed.

The experimental procedure was similar to that employed in the previous study on intact dogs (1). Each experiment began with a predecompression phase, during which the animal was kept at ground level. During this time, two blood samples were drawn, at an interval of 20 minutes, to establish initial values for the various components studied. These and all subsequent samples were taken from the jugular vein, using heparin as an anticoagulant. Ten minutes after the second blood sample had been drawn, the animal was placed in a decompression chamber and subjected to a simulated altitude of 30,000 feet (225.6 mm. Hg) for 90 minutes. At the end of each 30-minute period of this

Received December 12, 1956.

[1] This research was supported in part by the United States Air Force under Contract No. AF 41(657)-21, monitored by the School of Aviation Medicine, USAF, Randolph Air Force Base, Texas.

[2] Preliminary report, *Fed. Proc.* **15**: 62. 1956.

decompression phase a blood sample was drawn. Following decompression, the animal was returned to ground level where it remained throughout the postdecompression phase which terminated the experiment. This phase was usually two hours in duration, with a blood sample taken at the end of each hour. In control experiments, the dogs were subjected to the above procedure except that they were not actually decompressed during the second phase.

Plasma sodium and potassium concentrations were determined by flame photometry, using the internal standard method. Hematocrit values were read in Wintrobe hematocrit tubes after 25 minutes of centrifugation at about 2800 RPM. The number of circulating eosinophils per cu. mm. of blood was determined from a differential count of leukocytes made upon a blood film stained with Wright's stain.

In three of the dogs, which have died since the beginning of this program, autopsy has failed to reveal any trace of adrenal glands. In the remaining animal, absence of functional adrenal tissue is indicated by the appearance of classical symptoms of adrenal cortical insufficiency when supportive therapy is withdrawn for a few days. The results of studies of hematocrit values and eosinophil counts carried out during the experiments were also compatible with the view that adrenalectomy was complete. It was found that the increased hematocrit and the eosinopenia, which characteristically result in intact dogs subjected to a similar degree of decompression stress (1), failed to occur in any of the animals used in the present investigation.

RESULTS

Effects of Acute Decompression Stress on Adrenalectomized Dogs Maintained on Cortisone

Table 1 shows the effects of decompression to a simulated altitude of 30,000 feet on plasma potassium and plasma sodium in a series of 17 experiments carried out while the dogs were being maintained on cortisone therapy. In each case recorded, the experiment was continued for one hour after termination of decompression, and in 12 it was continued for two hours. The series includes six experiments upon Dog 31, five each upon Dogs 34 and 50, and one upon Dog 33, which succumbed during the course of a subsequent experiment in which it was exposed to severe decompression (50,000 feet). Consequently, data pertaining to this animal are limited to a single experiment.

The data reveal that, without exception, the plasma potassium concentration decreased during the periods of decompression. The major change clearly occurs during the first 30-minute period, although in most instances the lowest level is not reached until the end of the third period. An exception to this general effect is seen in the case of Experiment 189, Dog 34, where the potassium reached its lowest level during the first period and then rose slightly during the two subsequent periods. During the first hour of the postdecompression phase the potassium characteristically rises toward the initial level (except in Expts. 189, Dog 34, and 196, Dog 50). However, even in the 12 experiments carried through the second hour of the postdecompression phase, complete restoration of the initial plasma potassium concentration was observed in only one case (Expt. 192, Dog

34). It would, therefore, appear that in these adrenalectomized animals recovery of the plasma potassium concentration is somewhat slower than that observed in intact dogs where the initial level is usually reestablished within less than two hours after termination of decompression to 30,000 feet (1).

As indicated by the values in the lower part of the table, the mean plasma potassium concentration for the 17 experiments fell from a pre-decompression value of 5.33 m.eq./L. to a value of 4.43 m.eq./L. during

TABLE 1. EFFECTS OF ACUTE DECOMPRESSION STRESS (30,000 FEET) ON PLASMA POTASSIUM AND PLASMA SODIUM IN ADRENALECTOMIZED DOGS MAINTAINED ON CORTISONE

(m.eq./L. Plasma)

Dog No.	Expt. No.	Predecompression*		Decompression†						Postdecompression‡			
				Sample 1		Sample 2		Sample 3		Sample 1		Sample 2	
		K	Na	K	Na	K	Na	K	Na	K	Na	K	Na
31	161	5.75	154.5	4.92	154.2	4.60	155.0	4.37	155.0	4.64	155.2		
	166	7.65	142.4	5.15	142.0	4.77	141.6	4.77	142.2	5.35	142.2		
	199	4.71	129.4	4.04	130.0	3.85	130.6	3.75	130.0	3.98	130.4	4.08	130.4
	201	4.05	115.2	3.57	115.0	3.55	115.4	3.43	115.6	3.50	115.8	3.59	115.6
	204	4.88	102.9	3.80	101.8	3.61	100.6	3.64	101.0	3.93	101.8	4.12	101.2
	213	5.05	148.6	4.35	148.4	4.20	147.8	4.15	148.6	4.88	148.6	4.87	148.8
33	127	5.86	168.3	4.70	167.4	4.24	166.4	4.21	166.4	5.09	166.0		
34	159	4.27	164.1	3.70	163.8	3.39	164.0	3.43	163.8	3.78	164.0		
	168	4.66	161.2	4.17	161.4	3.96	160.8	3.88	162.2	4.22	161.4		
	186	5.23	126.6	4.18	126.4	4.26	126.8	4.08	126.4	4.45	126.2	4.15	126.6
	189	5.15	147.2	4.64	147.0	4.98	147.4	4.94	147.2	4.83	147.4	4.78	147.2
	192	4.51	159.3	4.31	160.2	4.15	160..	4.15	159.4	4.50	159.2	4.54	159.4
50	196	7.38	134.4	6.44	135.0	6.32	134.8	5.90	135.0	5.61	135.0	6.65	134.8
	200	4.54	120.4	3.80	121.2	3.72	120.2	3.60	121.2	4.20	121.4	4.42	121.0
	202	4.95	110.3	3.99	110.4	3.65	110.0	3.36	110.0	3.87	110.6	4.22	110.4
	205	5.76	95.8	4.70	96.0	4.58	95.8	4.41	95.8	5.04	95.8	5.60	96.0
	208	6.14	141.3	4.93	141.4	4.63	141.4	4.50	142.0	4.87	142.2	5.75	142.2
Mean		5.33	136.6	4.43	136.6	4.26	136.4	4.15	136.6	4.51	136.7		
S.E.§		± .24	±5.4	± .18	±5.3	± .18	±5.4	± .16	±5.4	± .15	±5.3		
Undecompressed controls													
Mean (7 expts.)		5.38	149.4	5.55	149.4	5.55	149.8	5.43	149.8	5.05	149.4	5.10	149.7
S.E.		± .19	±2.7	± .26	±3.1	± .16	±2.9	± .19	±2.6	± .14	±3.0	± .18	±2.5

* Predecompression values represent averages calculated from two blood samples taken 20 minutes apart.
† Decompression samples 1, 2 and 3 taken after 30, 60 and 90 minutes at 30,000 feet.
‡ Postdecompression samples 1 and 2 taken 1 and 2 hours after return to ground level.
§ Standard error of mean.

the first period of decompression and then to slightly lower values of 4.26 and 4.15 m.eq./L. during the second and third periods. These decreases are of the order of 16.9, 20.1 and 22.1%, respectively, below the predecompression concentration. In the seven control experiments summarized at the bottom of Table 1, no decrease was observed in the potassium level during the periods corresponding to decompression. It is, therefore, apparent that the decompression and not the stress of handling, withdrawal of blood, etc., accounts for the characteristic hypokalemia in the experimental series.

Unlike potassium, the plasma sodium concentration failed to show any marked or consistent change during or after exposure of the cortisone-maintained adrenalectomized dogs to a simulated altitude of 30,000 feet

(Table 1). A similar stability of the plasma sodium of intact dogs under comparable conditions has been demonstrated in a previous communication (1).

Effects of Acute Decompression Stress on Adrenalectomized Dogs Maintained on DCA

As shown in Table 2, the effects of decompression on the adrenalectomized dogs during DCA therapy were similar to those obtained while they were receiving cortisone. With the exception of two experiments, in which the predecompression value was very low (Expts. 198 and 203, Dog

TABLE 2. EFFECTS OF ACUTE DECOMPRESSION STRESS (30,000 FEET) ON PLASMA POTASSIUM AND PLASMA SODIUM IN ADRENALECTOMIZED DOGS MAINTAINED ON DCA
(m.eq./L. Plasma)

Dog No.	Expt. No.	Predecompression*		Decompression†						Postdecompression‡			
				Sample 1		Sample 2		Sample 3		Sample 1		Sample 2	
		K	Na	K	Na	K	Na	K	Na	K	Na	K	Na
31	181	4.27	166.7	3.70	166.9	3.70	167.0	3.69	166.8	3.81	166.8		
	184	5.76	148.9	4.61	148.2	4.02	148.4	3.95	149.6	4.42	149.4		
	188	5.12	144.9	4.53	145.6	3.98	145.4	3.89	145.6	4.43	145.0	4.45	145.6
	191	5.27	152.4	4.31	152.0	4.65	152.2	4.39	152.5	5.00	152.2	4.88	152.0
	194	4.22	153.5	3.54	153.2	3.65	153.4	3.65	153.2	3.97	153.0	3.87	153.4
34	195	4.15	159.2	3.58	158.6	3.33	158.0	3.40	158.4	3.47	158.8	3.97	158.6
	198	3.36	110.7	3.63	110.6	3.35	111.0	3.21	110.4	2.88	110.8	2.90	110.8
	203	2.71	122.3	2.71	123.0	2.70	123.0	2.71	122.8	2.61	122.6	2.71	122.2
	206	3.82	107.5	3.50	107.2	3.51	107.5	3.55	107.6	3.41	107.5	3.48	107.5
	209	4.22	147.5	3.61	147.0	3.51	147.2	3.47	148.0	3.73	148.2	3.76	147.8
	212	3.66	152.8	3.53	153.6	3.41	153.2	3.38	153.8	3.18	153.6	3.48	153.6
50	169	4.26	169.0	3.13	169.8	3.22	169.2	3.22	171.0	3.88	170.4		
	173	4.21	161.6	3.54	162.0	3.54	161.2	3.84	161.4	4.16	161.6		
	182	4.85	150.6	4.05	150.6	4.01	151.6	4.01	150.6	4.84	151.0	4.98	
	185	4.17	156.4	3.35	156.2	3.31	156.8	3.39	156.6	3.71	157.0	3.93	157.0
	187	4.32	148.9	3.50	149.1	3.44	149.1	3.18	149.2	3.88	149.4		
Mean		4.27	147.1	3.68	147.1	3.58	147.1	3.56	147.3	3.84	147.3		
S.E.§		±.19	±4.4	±.12	±4.6	±.12	±4.6	±.10	±4.7	±.16	±4.6		
Undecompressed controls													
Mean (5 expts.)		4.36	150.3	4.23	150.4	4.22	150.4	4.16	150.4	4.05	150.5	4.14	150.3
S.E.		±.39	±1.4	±.35	±2.5	±.34	±0.6	±.29	±1.9	±.30	±1.3	±.48	±2.3

*†‡§ As in Table 1.

34), plasma potassium concentration decreased during the first period of exposure to 30,000 feet and remained depressed during the two subsequent periods. The mean concentration decreased from a predecompression value of 4.27 m.eq./L. to levels of 3.68, 3.58 and 3.56 during the first, second and third decompression periods, respectively. Although these decreases are not as striking as those observed in the cortisone series, they are found to be highly significant ($P < .001$ in each case) and are of the order of 13.8, 16.2 and 16.6%. In the majority of instances (11 of the 16 experiments) the potassium concentration tended to rise again during the first hour of the postdecompression phase. In only one case was the initial level reestablished by the end of this time, however, and in only two of 11 experiments was it reestablished by the end of the second hour of postdecompression. As in the cortisone series, recovery of the initial potassium level

following decompression appears to be somewhat slower than in intact dogs (1).

Further inspection of Table 2 reveals that the plasma sodium concentration manifested its characteristic stability during and following decompression.

Effects of Acute Decompression Stress on Dogs in Moderate Adrenal Insufficiency

As a supplement to the preceding studies, a few similar experiments were carried out on two dogs in which a state of moderate adrenal insufficiency was allowed to develop by withholding supportive therapy for from 4 to 8 days following routine maintenance upon cortisone. Contrary

TABLE 3. EFFECTS OF ACUTE DECOMPRESSION STRESS (30,000 FEET) ON PLASMA POTASSIUM OF ADRENALECTOMIZED DOGS IN MODERATE INSUFFICIENCY

Dog. No.	Expt. No.	Days insuff.	Plasma K concentration—m.eq./L.					
			Predecompression*	Decompression†			Postdecompression‡	
				Sample 1	Sample 2	Sample 3	Sample 1	Sample 2
31	214	5	7.10	5.47	5.13	4.84	5.34	5.66
50	245	4	4.06	3.02	3.22	3.03	3.61	3.94
	222	5	5.82	5.04	4.43	4.37	5.32	6.19
	233	6	7.70	6.59	7.31	6.88	8.13	7.78
	216	8	7.63	7.13	6.89	6.68	7.76	8.12

*†‡ As in Table 1.

to expectation, it was found that the animals tolerated the altitude of 30,000 feet remarkably well in this condition. They remained conscious and manifested no particular distress throughout the entire procedure. Table 3 records the plasma potassium changes observed in five complete experiments, one involving Dog 31 and the other four involving Dog 50, which is the only one of the original animals surviving at the present time. In each case, the concentration decreased in the usual manner during the first 30-minute period of decompression and remained depressed during the two subsequent periods. During the first hour of the postdecompression phase it returned toward or, in two instances (Expts. 233 and 216, Dog 50), actually exceeded the initial level. As usual, the plasma sodium concentration failed to show any consistent change during or after decompression.

DISCUSSION

The present study shows that adrenalectomized dogs, maintained on cortisone or DCA, tolerate exposure to an altitude of 30,000 feet for 90 minutes as well as do intact dogs. At the end of this time the animals were fully conscious, alert and able to stand. Two dogs in which moderate adrenal insufficiency was allowed to develop by the withdrawal of supportive therapy also survived this altitude without apparent ill effects.

These facts are surprising in view of the well-known inability of most adrenalectomized laboratory animals to withstand stress (4, 5). Kline (2) reported that cats adrenalectomized 24 hours prior to decompression could survive an average of only 14 minutes at 28,000 feet. Pratt *et al.* (3), however, observed no difference between intact and adrenalectomized rats maintained upon 1% NaCl in their ability to withstand the stress of exposure to 30,000 feet for 30 minutes.

Under the conditions of the present study, adrenalectomized dogs were found to manifest the characteristic decrease in plasma potassium concentration seen in the intact dog (1), cat (2) and rat (3, 6) upon exposure to 30,000 feet for 30 minutes or more. It therefore appears that, in the dog, the hypokalemia of moderate decompression stress does not depend upon the presence of functioning adrenal glands. Since the response continues to occur in these animals when supportive therapy has been withheld for several days, it would seem valid to make the additional assumption that it does not depend upon the presence of circulating cortisone or DCA. These results are compatible with those obtained by McQuarrie *et al.* (7) who found that in adrenalectomized dogs, given cortin only on an emergency basis, hypoxia produced by breathing an atmosphere of 5% oxygen caused "a fairly marked decrease in serum potassium".

In a recent study on unanesthetized, chronically adrenalectomized rats, Pratt *et al.* (3) found that the plasma potassium concentration remained unchanged at the end of 30 minutes of decompression to a simulated altitude of 30,000 feet, although a mean fall of 15.9% had been observed in intact rats under the same conditions. From these results it is apparent that in the rat, unlike the situation in the dog, the hypokalemic response to altitude is mediated by the adrenals. Kline (2) has reported that adrenalectomized cats fail to manifest a significant decrease in plasma potassium concentration upon exposure to 28,000 feet for 90 minutes. In view of the close phylogenetic relationship between the cat and dog, the apparent difference in the potassium response of the two species to altitude after adrenalectomy is somewhat surprising. It should be noted, however, that the negative results reported for the cat were obtained upon anesthetized animals from which the adrenals had been removed only two hours before decompression (2). So far as we are aware, comparable studies have not been carried out on unanesthetized, chronically adrenalectomized cats. It has been our experience, with dogs, that the combination of anesthesia and postoperative trauma tends to diminish, and sometimes to abolish, the potassium response to an altitude of 30,000 feet. The extent to which these factors, rather than absence of the adrenal glands, might have operated to prevent the hypokalemic response in Kline's study cannot be ascertained, but it would seem advisable to bear in mind the possibility that the discrepancy between the results obtained to date with the cat and dog may reflect differences in experimental procedure rather than a fundamental species

difference in the rôle of the adrenal glands in mediating the potassium response to altitude.

In attempting to explain the mechanism by which the plasma potassium reponse is mediated in dogs exposed to acute decompression stress, it would seem appropriate to consider the possible significance of acid-base changes of respiratory origin. The recent literature contains several reports which show that the respiratory alkalosis resulting from hyperventilation produces hypokalemia (8, 9, 10, 11), and studies in our laboratory indicate that a respiratory alkalosis characteristically accompanies the decrease in plasma potassium in dogs exposed to an altitude of 30,000 feet. These facts would indicate the advisability of examining by appropriate experimentation the hypothesis that, in the dog, the hypokalemia of decompression stress, like that occurring during hyperventilation, is due to the development of respiratory alkalosis.

SUMMARY

Some effects of decompression to a simulated altitude of 30,000 feet for 90 minutes have been studied upon unanesthetized, bilaterally adrenalectomized dogs maintained on cortisone or DCA, or in moderate adrenal insufficiency. Plasma potassium concentration consistently decreased by the end of the first 30 minutes and remained depressed for the duration of decompression. This effect is similar to that observed in intact dogs under comparable conditions. It is therefore concluded that, in this species, the hypokalemic response to decompression stress is not mediated by the adrenal glands. As in intact dogs, the plasma sodium concentration remained unchanged during decompression.

Acknowledgments

The authors wish to express their gratitude to Dr. George Schimert for performing the adrenalectomies and to Miss Carolyn F. Hendrickson for her technical assistance.

REFERENCES

1. Ferguson, F. P. and D. C. Smith: *Am. J. Physiol.* **173:** 503. 1953.
2. Kline, R. F.: *Fed. Proc.* **11:** 84. 1952. See also Thesis submitted to the Faculty of the Graduate School of the University of Maryland, 1951.
3. Pratt, A. J., D. C. Smith and F. P. Ferguson: *Endocrinology* **57:** 450. 1955.
4. Swingle, W. W. and J. W. Remington: *Physiol. Rev.* **24:** 89. 1944.
5. Sayers, G.: *Physiol. Rev.* **30:** 241. 1950.
6. Sussman, K. E., A. J. Pratt, D. C. Smith and F. P. Ferguson: *Bull. Sch. Med., Univ. Maryland* **38:** 164. 1953.
7. McQuarrie, I., M. R. Ziegler, W. E. Stone, O. H. Wangensteen and C. Dennis: *Chinese Med. J.* **58:** 26. 1940.
8. Stanbury, S. W. and A. E. Thomson: *Clin. Sci.* **11:** 357. 1952.
9. Elkinton, J. R., R. B. Singer, E. S. Barker and J. K. Clark: *J. Clin. Investigation* **34:** 1671. 1955.
10. Scribner, B. H. and J. M. Burell: *J. Clin. Investigation* **34:** 919. 1955.
11. Giebisch, G., L. Berger and R. F. Pitts: *J. Clin. Investigation* **34:** 231. 1955.

EFFECT OF SYNTHETIC ANALOGUES OF HYDROCORTISONE ON THE BLOOD PRESSURE OF ADRENALECTOMIZED RATS ON SODIUM RESTRICTION

ABBIE I. KNOWLTON, EMILY N. LOEB AND HERBERT C. STOERK

From the Department of Medicine, Columbia University College of Physicians and Surgeons, and the Presbyterian Hospital, New York, N. Y., and the Merck Institute for Therapeutic Research, Rahway, New Jersey

PREVIOUS experimentál work has established that several of the adrenal steroids may produce hypertension: desoxycorticosterone (DOC) (1, 2, 3, 4), cortisone (5, 6) and hydrocortisone (7). It seems likely that more than one mechanism is involved in the action of these steroids upon the blood pressure. That of desoxycorticosterone is conditioned by the amount of sodium available, i.e., the development of hypertension is accentuated by a liberal sodium intake (4) and can be prevented by sodium restriction (8). In contrast, the action of cortisone is not influenced by variation in the sodium content of the diet (6) and the hypertension induced by hydrocortisone is not agumented by a high sodium intake or by reduction in renal mass (9). This suggests that both cortisone and hydrocortisone produce their effect upon the blood pressure through some mechanism which differs from that invoked by desoxycorticosterone.

In the last three years numerous analogues of eleven oxygenated steroids have been synthesized, several of which possess extraordinary physiological activity (10, 11, 12, 13). In a number of these compounds the modification in structure has resulted in a strikingly greater activity with respect to liver glycogen than hydrocortisone, while the capacity to modify sodium metabolism has varied widely and independently of the glycogen effect. Table 1 outlines the reported results obtained in a number of assays with certain of these compounds, the activity of DOC being taken as the standard of reference and arbitrarily considered as 1 in assays measuring sodium retention, while the activity of cortisone has been similarly treated in assays based on liver glycogen determinations.

The effect of these new steroids on the blood pressure has not been extensively investigated, although Friedman, Friedman and Nakashima (19) and also Selye (20) have reported that the administration of 9-α-chlorohydrocortisone may result in hypertension. In the following an attempt has been made to compare the potency of various synthetic steroids with re-

Received December 21, 1956.

TABLE 1. RELATIVE ACTIVITY OF SYNTHETIC STEROIDS AS REPORTED IN THE LITERATURE

Steroid	Capacity to induce sodium retention		Activity with respect to liver glycogen
	A. Acute studies	B. Life maintenance studies	
Hydrocortisone	"very slight" (15)		1(15)
Cortisone		±.05–.025*(14)	1
1-dehydro-hydrocortisone 1-dehydro-cortisone	"very slight" (15)		3–4(12), 2.9(15), 3–4(18)
9α-fluorohydrocortisone	>1(16), 5(15), 4.7(21)	5–10*(17), <1(16), 1(22)	10.7(11), 12.6(15)
9α-chlorohydrocortisone	10.8(16), 3.3(21)	±2*(17), 10–20(16), 10(22)	~4(10)
1-dehydro-9α-fluorohydrocortisone	4.6(15)		50(15), 25(13)
Desoxycorticosterone	1	1	

Figures in parentheses refer to the bibliography.
* Extrapolated from minimum maintenance dosages.

gard to their blood pressure elevating action in adrenalectomized rats restricted as to their sodium intake. This regimen was selected as one which would prevent the appearance of "DOC-like" hypertension but not the possible development of a rise in pressure such as accompanies the administration of "cortisone-like" steroids. It seemed possible that such a study might throw light on the nature of the mechanism by which hydrocortisone and cortisone induce hypertension.

METHODS

The data were accumulated in thirteen successive series of observations and comprised a total of 521 young Sprague-Dawley rats which were divided into groups of six to eight, as shown in the tables. The average weight per group ranged from 116 to 143 grams at the start of the experimental period, at which time all animals were adrenalectomized and placed on a low sodium diet.[1] The rats were injected subcutaneously over a period of two weeks (six days a week). The steroids were administered in oil, either as the free alcohol or in the acetate form. The results obtained with cortisone and hydrocortisone were indistinguishable and have been combined in the tables and charts for the sake of brevity. For similar reasons, the data pertaining to 1-dehydro-hydrocortisone and 1-dehydrocortisone have also been combined. Groups of six to ten normal animals at a time were placed on the low sodium diets and observed along with the experimental groups, comprising the "intact controls" included in the tables. All animals were weighed twice weekly and blood pressure readings were made at the end of a two-week experimental period (6). At this time the rats were sacrificed by exsanguination from the incised heart after preliminary barbiturate anesthesia. The blood obtained was separated, and sera from 2–4 animals combined for analyses of sodium and potassium by flame photometry.

RESULTS AND DISCUSSION

Effect of steroids on weight:—The effect of these compounds upon the body weight may be seen in Table 2. As observed previously (24) no

[1] Details of this synthetic diet have been published previously (23). In a few series, a natural low sodium diet of similar protein composition was substituted (24). Analyses of both diets indicated they contained similar concentrations of sodium (0.01 to 0.02%), and potassium (0.28 to 0.3%). As the results observed on both diets were comparable, the data have been combined in the charts and tables.

TABLE 2. EFFECT OF STEROIDS ON GROWTH OF ADRENALECTOMIZED RATS ON SODIUM RESTRICTION

Daily steroid dosage in mg.	Intact controls	Hydrocortisone Cortisone	1-dehydro-F 1-dehydro-E	9α-F1F	9αC1F	1-dehydro-9α-F1F	DOC
	Average weight change in grams at the end of two weeks						
0	+36(38)						
0.008						−4*	
0.01				−13(4)**	+15		
0.03				−2		−1	all*
0.1		all*			+25		+16
0.12						+5 +15 −14 +20	+7(5)***
0.2		−12(3)*****		+15*			
0.3		−20(4)****					
0.33				+18 −7 +11			
0.4						−18*	
0.5		−27(3)*****	all*				
0.8			−34 −31 −29				
1.0		−17(4) −22 −9(5) −7	−43(4)**** −17	−37(4)** −34 −22** +1	−7	−24(1)*******	+8
1.2						−39(1)*******	
2.0		−23*	−37*				
2.5		−23 −26 −30 −17		−44(5)**			
3.0		−29**					+49

Figures in parentheses indicate number of rats on which average is based. When no parenthesis appears the number of animals is 6 to 8.
* Each * represents an animal which died during the experimental period.

dosage of hydrocortisone or cortisone promoted weight gain, while the animals receiving the higher dosages experienced severe weight losses which approximated one-quarter of their initial body weight. With either 1-dehydro-hydrocortisone or 1-dehydro-cortisone there was, similarly, no level at weight loss was prevented. No rats survived on the 0.5 mg. dose, and above this level those that were able to live out the two-week experimental period exhibited even more striking weight losses than the groups on the parent compound. Many of these rats appeared in extremely poor condition. Growth approached normal with appropriate doses of 9-α-fluorohydrocortisone, 9-α-chlorohydrocortisone, and 1-dehydro-9-α-fluorohydrocortisone. With higher doses, however, these compounds resulted in as profound weight losses as did hydrocortisone, cortisone, 1-dehydrohydrocortisone, and 1-dehydro-cortisone.

Desoxycroticosterone, in the ranges of dosage explored in this study, consistently promoted growth.

TABLE 3. SERUM SODIUM CONCENTRATIONS OF ADRENALECTOMIZED RATS AFTER TWO WEEKS OF SODIUM RESTRICTION
mEq/L.

Daily steroid dosage, mg.	Intact controls	Steroids					
		Hydrocortisone and cortisone	1-dehydro-F 1-dehydro-E	9-α-FIF	9α-CIF	1-dehydro-9α-FIF	DOC
0	139.3(30)*						
0.008						122(7)	
0.01				120(4)	133(8)		
0.03				120(8)		120(8)	
0.12						139.9(32)	
0.2				136.0(7)			
0.33				143.1(24)			
0.4						144.5(7)	
0.8			137.0(20)				
1.0		133.7(17)	136.0(8)	146.0(22)	143(8)	136.0(1)	140.0(8)
2.5		143.2(29)		146.9(4)			

* Figures in parentheses indicate number of individual rats from which sera was drawn and pooled in groups o 2 to 4 animals for analyses.

Effect of steroids on serum electrolytes:—Under the conditions of rigid restriction of sodium intake, none of the the steroids examined led to striking elevation of the serum sodium concentration or reduction in potassium (Tables 3 and 4). Those compounds with marked sodium retaining capacities, 9-α-fluorohydrocortisone, 1-dehydro-9-α-fluorohydrocortisone, and 9-α-chlorohydrocortisone, did permit the maintenance of normal concentrations of both these ions at remarkably low doses. In relatively higher doses, i.e., 0.4 mg. daily, the 1-dehydro-9-α-fluoro-compound was accompanied by borderline elevation in the sodium level while the 9-α-fluoro- steroid led to definitely increased values of sodium as well as a suggestive lowering of the potassium concentration at a dose of 1 mg. daily.

Effect of steroids on blood pressure:—The blood pressure readings are set forth in Table 5, and in Figure I the dose response curves are presented. No curve could be computed for 1-dehydro-hydrocortisone and 1-dehydrocortisone as the limited range of dosage over which these steroids permitted survival was too narrow to afford sufficient data (see Table 5).

TABLE 4. SERUM POTASSIUM CONCENTRATION OF ADRENALECTOMIZED RATS AFTER TWO WEEKS OF SODIUM RESTRICTION
mEq/L

Daily steroid dosage mg.	Intact controls	Steroids				
		Hydrocortisone and Cortisone	1-dehydro-F 1-dehydro-E	9α-FIF	9αCIF	1-dehydro-9α-FIF
0	5.2(10)*					
0.01					7.0(8)	
0.12						4.1(8)
0.33				4.3(8)		
0.8			5.6(8)			
1.0		6.3(4)		3.3(8)	5.2(1)	
2.5		5.7(8)				

* Figures in parentheses indicate number of individual rats from which sera was drawn and pooled in groups of 2 to 4 animals for analyses.

TABLE 5. EFFECT OF STEROIDS ON BLOOD PRESSURE OF ADRENALECTOMIZED RATS ON SODIUM RESTRICTION

Daily steroid dosage in mg.	Intact controls	Hydrocortisone Cortisone	1-dehydro-F 1-dehydro-E	9α-F1F	9α-C1F	1-dehydro-9α-F1F	DOC
0	145± 12.7(38)†						
0.008						101± 18.4(5)***	
0.01				66± 21.7(3)***	121± 13.7		
0.03				105± 20.8		120± 12.1	all*
0.1		all*			149± 9.0		100± 25.8
0.12						153± 11.0 165± 9.9 174± 13.1 158± 9.2	99± 17.7(4)****
0.2		all*		163± 11.3*(7)			
0.3		all*					
0.33				157± 14.1 176± 14.6 161± 10.2			
0.4						160± 12.9(6)**	
0.5		all*	all*				
0.8			101± 8.7(4)**** 173± 13.2 149± 17.0*(7)				
1.0		172± 7.6(4)‡ 156± 23.3 166± 16.1(5) 137± 8.3	130± 22.8(3)**** 151± 10.8	166± 15.6(5)* 176± 32.8(7) 135± 15.8**(6) 166± 12.7*(7)	169± 9.6	all*	120± 14.8(7)*
1.2						138(1)*******	
2.0		177± 18.4	159± 21.0*(7)				
2.5		184± 15.2 192± 20.8(6) 181± 8.8 181± 15.7		148± 21.9(4)***			
3.0		189± 10.6**(6)					150± 7.4

† Each number represents the average of blood pressure readings of eight rats. When average is based on more or less than this, the number is indicated in parentheses. Standard deviation which follows average determined

* Each * represents an animal which died during the experimental period or, if surviving, had an unobtainable blood pressure.

$$\sqrt{\frac{\Sigma(x-\bar{x})^2}{n-1}}$$

* Represents number of deaths plus number with unobtainable blood pressure readings.

‡ When more than one number is listed at a given dosage, each indicates a separately studied group of rats.

In computing the curves for the remaining compounds only those doses were considered which permitted survival of all or, in one case, of all but one of the rats per group. Examination of Figure 1 shows that the slope of the line characterizing hydrocortisone and cortisone (73.8 S.E. 12.0) is distinctly different from that describing desoxycorticosterone (31.9 S.E. 5.9). This difference is significant at a 5% level.[2]

If the blood pressure response varied only with the potency which the compound possessed as measured by liver glycogen assays, the slopes of the lines from all the synthetic steroids should parallel the hydrocortisone line. If, however, the "log. dose" response varied only with the capacity of

[2] The authors are much indebted to Dr. John W. Fertig and Miss Else Berquo of the School of Public Health and Administrative Medicine of the Faculty of Medicine, Columbia University, for their assistance in the statistical analysis of the data.

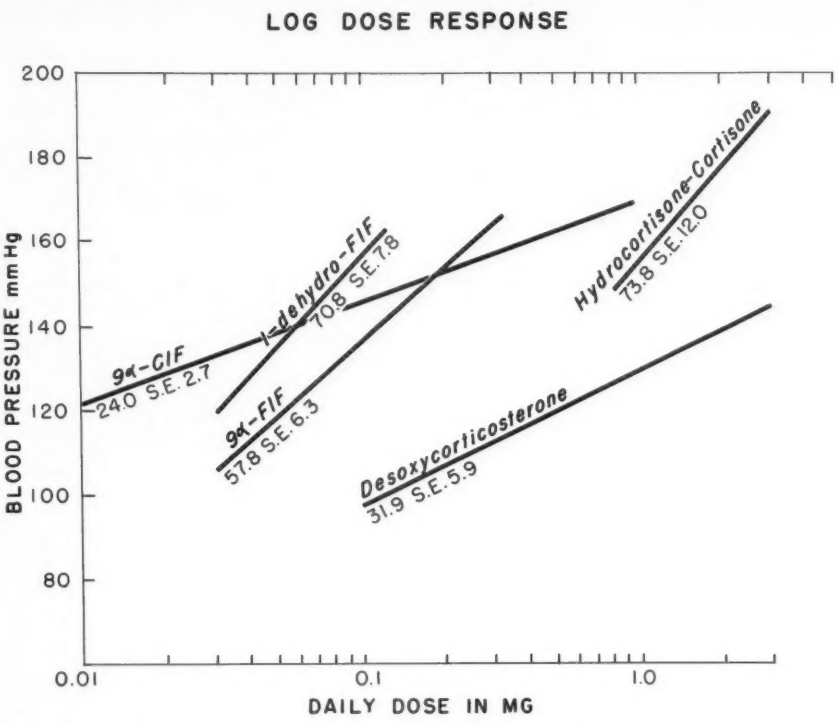


FIG. 1. Effect of steroids on blood pressure of adrenalectomized rats on sodium restriction.

the compound to retain sodium, the slopes of all should parallel the line describing DOC. The figure indicates that neither of these possibilities is the sole determinant. What is actually found is that the lines describing 1-dehydro-9-α-fluorohydrocortisone (70.8 S.E. 7.8) and 9-α-fluorohydrocortisone (57.8 S.E. 6.3) are not significantly different from the cortisone line, while that of 9-α-chlorohydrocortisone (24.0 S.E. 2.7) does not differ significantly from the DOC line. It is of interest that the two fluoro-substituted steroids possess 10 to 50 times greater potency in liver glycogen assays than hydrocortisone itself and one to five times more sodium retention than DOC (Table 1), while the chloro-compound's activity is four-fold that of hydrocortisone and three- to ten-fold that of DOC. In other words the ratio of liver glycogen activity to sodium retaining activity is higher in the fluoro as compared to the chlorosubstituted steroids.

The foregoing considerations led to the hypothesis that although the capacity of these several steroids to elevate the blood pressure under conditions of sodium restriction is intimately linked with that adrenal activity measured in liver glycogen assays, the sodium retaining activity,

under this special experimental regimen plays a permissive role. In this role it prevents, when present in sufficient quantity, death from sodium depletion, thus allowing the blood pressure elevating effect of the glycogen-linked adrenal activity to become manifest. According to this hypothesis, the slope of the line describing the blood pressure response to increasing doses for any given steroid is determined by the ratio of these two separate adrenal functions which are present in the steroid, rather than by either one alone.

TABLE 6. COMPARISON OF 9α-CHLOROHYDROCORTISONE WITH COMBINATIONS OF 1-DEHYDRO-HYDROCORTISONE AND DESOXYCORTICOSTERONE AS TO EFFECTS ON BLOOD PRESSURE, THYMUS WEIGHT AND SERUM ELECTROLYTE CONCENTRATIONS IN ADRENALECTOMIZED RATS ON SODIUM RESTRICTION

Group	Steroid	Daily steroid dosage in mg.	Terminal blood pressure, mm. Hg. Average S.D.	Average weight change, gm.	Average thymus weight, mg.	Serum sodium,* mEq./L.	Serum potassium,* mEq./L.
I	9α-C1F	0.01	124 ± 14.9	+20	528	126.3	8.3
Ia	1-dehydro-F	0.0075	132 ± 8.4	+26	459	133.8	8.3
	DOC	0.04					
II	9α-C1F	0.1	140 ± 7.5	+33	384	130.5	6.9
IIa	1-dehydro-F	0.075	136 ± 10.2	+25	381	131.5	6.9
	DOC	0.4					
III	9α-C1F	0.5	157 ± 12.6	+30	113	135.5	6.2
IIIa	1-dehydro-F	0.4	156 ± 9.5	+33	187	135.5	6.2
	DOC	2.0					
IV	9α-C1F	1.0	169 ± 6.6	+6	32	137.0	7.1
IVa	1-dehydro-F	0.75	168 ± 11.3	+19	87	140.0	5.9
	DOC	4.0					
V	9α-C1F	2.0	154 ± 13.2	−16	22	143.5	4.6
Va	1-dehydro-F	1.5	159 ± 7.3	−23	38	141.0	5.7
	DOC	8.0					

* Average of two determinations, each made on pooled sera from 4 rats.

In order to examine this possibility further an attempt was made reproduce the slope of the line describing the log. dose response of blood pressure of one of the synthetic steroids, 9-α-chlorohydrocortisone, by an appropriate combination of steroids, one with an exclusively sodium-retaining activity, and the other with an adrenal cortical activity essentially limited to its effect on liver glycogen. For this reason a mixture of desoxycorticosterone and 1-dehydro-hydrocortisone was selected which was estimated to duplicate the activities with respect to sodium and to liver glycogen present in 9-α-chlorohydrocortisone.

The results are charted in Table 6, and the dose response curves are in Figure 2. The slope of the line which can be drawn for the combination of the two steroids does not differ significantly from the slope of the line

describing 9-α-chlorohydrocortisone, although there is some suggestion of a lack of complete linearity as the points depart somewhat more from the line than can be explained by variation in individual rats. The similarity of the weight changes, thymus weights and the serum electrolyte concentrations at each dosage level (see Table 6) gives further evidence that this particular combination of DOC and 1-dehydro-hydrocortisone satisfactorily reproduced the effect of 9-α-chlorohydrocortisone.

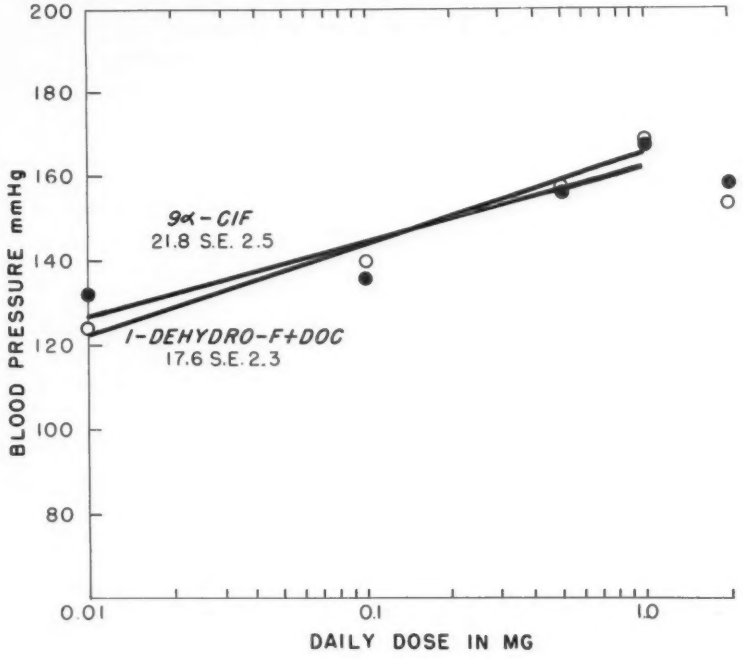


FIG. 2. Log dose response 9α-CIF vs. 1-dehydro-F+DOC.

SUMMARY

The blood pressure elevating action of a number of hydrocortisone analogues has been compared in adrenalectomized rats subjected to sodium deprivation. The administration of graded doses of each of the steroids resulted in progressive rises in blood pressure directly related to the level of the dose. The slope of the line describing this dose response was 31.9 for desoxycorticosterone and 73.8 for hydrocortisone. These two slopes differ significantly, reflecting the qualitatively different biological action of the two steroids. 9-α-fluorohydrocortisone and 1-dehydro-9-α-fluorohydrocortisone, compounds in which the ratio of hydrocortisone-like to desoxycorticosterone-like activity is high, give slopes which are not significantly different from that of hydrocortisone. Assuming they are

identical, the potency of 9-α-fluorohydrocortisone with regard to its blood pressure elevating effect is approximately five times, and that of 1-dehydro-9-α-fluorohydrocortisone, ten times that of hydrocortisone. 9-α-chlorohydrocortisone, in which the above mentioned ratio is low, gives a slope similar to desoxycorticosterone and, assuming these slopes are identical, has 40 to 50 times the potency of the latter.

It was possible to duplicate the dose response curve of 9-α-chlorohydrocortisone by a mixture of 1-dehydro-hydrocortisone and desoxycorticosterone in which the ratio of the activity as measured by liver glycogen assay to the activity as regards sodium retention was calculated to be the same as the proportion of these activities present in 9-α-chlorohydrocortisone.

Acknowledgment

The authors wish to express their gratitude to Tatania Budzilovich, Theodore Bielinski, Ritzu Arison and Josephine Hoban for technical assistance.

REFERENCES

1. Kuhlmann, D., C. Ragan, J. W. Ferrebee, D. W. Atchley and R. F. Loeb: *Science* **90**: 496. 1939.
2. Ferrebee, J. W., C. Ragan, D. W. Atchley and R. F. Loeb: *J.A.M.A.* **113**: 1725. 1939.
3. Grollman, A., T. R. Harrison and J. R. Williams, Jr.: *J. Pharm. & Exp. Therap.* **69**: 149. 1940.
4. Selye, H., C. E. Hall and E. M. Rowley: *Canad. Med. Assn. J.*, **49**: 88. 1943.
5. Gaudino, N. M.: *Rev. Soc. Arg. Biol.* **20**: 470. 1944.
6. Knowlton, A. I., E. N. Loeb, H. C. Stoerk, J. P. White and J. F. Heffernan: *J. Exper. Med.* **96**: 187. 1952.
7. Friedman, S. M., C. L. Friedman and M. Nakashima: *Endocrinology* **51**: 401. 1952.
8. Knowlton, A. I., E. N. Loeb, H. C. Stoerk and B. C. Seegal: *J. Exper. Med.* **85**: 187. 1947.
9. Friedman, S. M., C. L. Friedman and M. Nakashima. *Endocrinology* **53**: 633. 1953.
10. Fried, J. and E. F. Sabo: *J. Am. Chem. Soc.* **75**: 2273. 1953.
11. Fried, J. and E. F. Sabo: *J. Am. Chem. Soc.* **76**: 1455. 1954.
12. Hertzog, H. L., A. Nobile, S. Tolksdorf, W. Charney, E. B. Hershberg, P. L. Perlman and M. M. Pechet: *Science* **121**: 176. 1955.
13. Hirschmann, R. F., R. Miller, R. E. Beyler, L. H. Sarett and M. Tishler. *J. Am. Chem. Soc.* **77**: 3166. 1955.
14. Swingle, W. W., R. Maxwell, M. Ben, C. Baker, S. J. LeBric and M. Eisler: *Endocrinology* **55**: 813. 1954.
15. Stafford, R. O., L. E. Barnes, B. J. Bowman and M. M. Meinzinger: *Proc. Soc. Exp. Biol. and Med.* **89**: 371. 1955.
16. Borman, A., F. M. Singer and P. Numerof. *Proc. Soc. Exp. Biol. & Med.*, **86**: 570. 1954.
17. Swingle, W. W., C. Baker, M. Eisler, S. J. LeBric and L. J. Bramnick. *Fed. Proc.* **14**: 150. 1955.
18. Perlman, P. L. and S. Tolksdorf. *Fed. Proc.* **14**: 377. 1955.

19. Friedman, S. M., C. L. Friedman and M. Nakashima. *Endocrinology* **57**: 10. 1955.
20. Selye, H.: *J. Clin. Endocrinol. and Metab.* **15**: 384. 1955.
21. Liddle, G. W., M. M. Pechet and F. C. Bartter. *Science* **120**: 496. 1954.
22. Llaurado, J. G.: *Proc. Univ. Otago* **33**: 2. 1955.
23. Stoerk, H. C., A. I. Knowlton and E. N. Loeb. *Proc. Soc. Exp. Biol. & Med.*, **85**: 627. 1954.
24. Knowlton, A. I., E. N. Loeb and H. C. Stoerk. *J. Clin. Invest.* **32**: 1175. 1953.

THE INFLUENCE OF THE PARATHYROIDS ON BLOOD CALCIUM LEVELS AND SHELL DEPOSITION IN LAYING HENS[1,2]

D. POLIN[3] AND P. D. STURKIE

Rutgers University, New Brunswick, New Jersey

EFFECTS of parathyroidectomy on the deposition of shell calcium in the fowl have not been determined. Previous studies have been concerned with the function of the parathyroid glands in adult male or immature ducks (1, 2) and pigeons (3, 4). Some effort was directed toward correlating changes in bone during the egg formation cycle of laying pigeons (5) and with parathyroid histology in laying hens (6). In these preceding studies, parathyroidectomy of the laying female bird, at a time preceding or during shell deposition was not performed.

METHODS

Adult female chickens in their 1st and 2nd year of production were individually caged in 3-deck batteries, and given water and mash, *ad libitum*, except when starved; then only water was allowed. These hens were crossbreeds and leghorns.

Eggs were collected from the hens during a control period of 7 to 10 days before the starvation or operational regimen, and during the experimental period that followed. The eggs were weighed, broken open, and the egg shells washed under tap water. The shells were then dried at 105° C for 24 hours, cooled and weighed on an analytical balance. Shell membranes were included in the shell weight.

Blood samples were usually taken from the brachial vein, and at other times, by heart stab. A sample of 7 ml. provided sufficient plasma for total and diffusible calcium determinations. If only total calcium were desired, then 3 ml. of blood were removed. Heparin was used as the anticoagulant. Wintrobe tubes, spun for 30 minutes at 3000 r.p.m., were used to obtain hematocrit values. Calcium was determined in plasma and its ultrafiltrates by a method described elsewhere (7). Ultrafiltrates were obtained by a procedure similar to that employed by Moritz (8) with some modifications. Cellophane sacs, $\frac{3}{4}''$ open diameter, were prepared in 75–78 mm. lengths, and stoppered at one end with an "00" rubber stopper secured in place with a rubber band. This band was thoroughly washed with distilled water before use. The sac was washed inside and out with distilled water and hung upside down to drain and dry. Into a dry sac, 2.5 ml. of plasma were pipetted. A #1 one-hole rubber stopper, slightly moistened with distilled water,

Received December 21, 1956.

[1] This work has been supported by a fellowship grant from Limestone Products Corp. of America, Newton, N. J.

[2] Paper of the Journal Series, New Jersey Agricultural Exper. Station, Rutgers University, The State University of New Jersey, Department of Poultry Husbandry, New Brunswick.

[3] In partial fulfillment of Ph.D. thesis. Present address is Merck Institute for Therapeutic Research, Rahway, N. J.

was forced into the open end, and secured in place with another rubber band. The sac was then attached to the ultrafiltration apparatus by forcing the glass tubing of the apparatus through the one-hole stopper of the sac. A 50 ml. conical centrifuge tube covered the sac containing the plasma, and provided the receptable into which the ultrafiltrates were collected. A vacuum pump, with a self-regulating valve was used to maintain a negative pressure of 236 mm.Hg. The ultrafiltration apparatus operated at room temperature (approx. 20° C). Sufficient filtrate was obtained, after 16–18 hours, to provide duplicate calcium determinations on 0.5 ml. of fluid per determination. The ultrafilterable calcium, thus determined, is referred to as diffusible calcium, and the non-ultrafilterable calcium as non-diffusible calcium. The non-diffusible calcium was obtained by difference between the total and the diffusible calcium.

Parathyroidectomy. The chickens were anesthetized by injecting intravenously an aqueous solution of sodium pentobarbital, 30 mg./ml. Approximately 60–90 mg./bird allowed an operating time of 30–45 minutes. Respiratory failure, a frequent occurrence in anesthetized chickens was prevented with the use of a respiratory pump. A procedure (9) similar to that employed on the duck was used in removing the parathyroids from the chicken. The thyroids were not removed as previous investigators (10, 11) showed that no accessory parathyroid tissue was found within this gland. All birds dying during the experiment and those sacrificed were examined, visually, for the presence of parathyroid tissue. A parathyroidectomized bird was eliminated from the experiment if such tissue was found. In only 2 hens was tissue located. In most cases, birds were examined within 14–25 days after the operation; however, a few were not sacrificed until 32–40 days after parathyroidectomy. Sham operation consisted of all procedures up to but not including removal of the parathyroid. Instead, the glands were manipulated, the membranes punctured, or on occasion, one portion of a gland removed.

RESULTS

In laying hens, sampled at random during the daytime, there appeared to be a decrease in the diffusible calcium level at the time of shell deposition, although total calcium showed no change (Table 1). These results were corroborated in the next experiment in which a group of hens (Group A) sampled, first, when a soft egg reached the uterus, and then again 9–12 hours later during shell deposition, had significantly lower ($P < .01$) diffusible calcium levels, and essentially unchanged total calcium levels (Table 2). In another group of hens (B) the initial blood sample was removed from 4–15 hours before oviposition, and second sample taken $\frac{1}{2}$–2 hours after oviposition. No significant change occurred in the mean diffusible calcium values (Table 2), but many of the hens showed differences between first and second blood samples in excess of the variability attri-

TABLE 1. RELATIONSHIP OF TOTAL AND DIFFUSIBLE CALCIUM LEVELS IN LAYING HENS TO CALCIUM DEPOSITION ON THE EGG

	Soft egg in uterus	Hard shell egg in uterus	No. eggs in uterus
No. of Birds	6	19	23
Total calcium mg./100 ml.	24.2 ±0.7*	25.0 ±0.7	25.5 ±0.7
Diffusible calcium mg./100 ml.	7.1 ±0.2	6.5 ±0.2	7.5 ±0.2
% Diffusible calcium	29.2	26.0	29.4

* Mean ±S.E.M.

TABLE 2. PLASMA CALCIUM LEVELS IN HENS BEFORE, DURING AND AFTER SHELL DEPOSITION

a) Initial sample of Group A removed when soft egg palpated in oviduct. Second sample removed 9–12 hrs. later during shell deposition.
b) Initial sample of Group B removed when hard shell egg in uterus. Second sample removed ½–2 hrs. after oviposition.

Group	No. birds	Total calcium-mg./100 ml.			Diffusible calcium-mg./100 ml.			Non-diffusible calcium-mg./100 ml.		
		initial	2nd sample*	Dif.	initial	2nd sample	Dif.	initial	2nd sample	Dif.
A	10	19.0±.63	18.3±.80	−0.7	6.4±.23	5.1±.16	−1.3**	12.6±.58	12.7±.70	+0.1
B	12	22.7±.63	22.2±.65	−0.1	7.6±.23	7.1±.19	−0.5	15.2±.55	15.2±1.0	0.0

* Corrected on basis of hematocrit values.
** P = .01.

buted to the techniques that were used. Thus, the data indicated that the diffusible calcium level was not constant in laying hens, and that shell deposition, particularly during the early hours, significantly altered the level of this calcium fraction. It is important to note that the hens of Group A were sampled late at night when it is presumed that the calcium available for shell deposition came mostly from bone; whereas, the hens of Group B, sampled during the day, had access to feed and presumably had available both exogenous and endogenous calcium. The availability of feed calcium would appear important as 60–75% of the shell calcium has been calculated to come from ingested sources (12, 13).

Effect of of Parathyroidectomy. Marked decreases in plasma calcium occurred in parathyroidectomized laying hens. At the time of the operation 14 hens had a total calcium level of 27.0 ± 1.6 mg./100 ml. (mean ± s.e.). At 18–24 hours after the operation the calcium level was 10.9 ± 1.6 mg./100 ml., which was similar to the mean calcium level for all of the 27 parathyroidectomized hens (Table 3). Both diffusible and non-diffusible plasma calcium fractions were significantly ($P < .01$) below sham operated levels. In the sham operated hens, the non-diffusible calcium significantly decreased, an effect attributed to starvation (Table 3). Most of the parathyroidectomized hens did not eat for 2 days. Those that did eat had

TABLE 3. THE EFFECT OF STARVATION, SHAM PARATHYROIDECTOMY AND PARATHYROIDECTOMY ON BLOOD CALCIUM IN LAYING HENS. CALCIUM ANALYZED IN 18–24 HOUR BLOOD SAMPLES

	Total calcium, mg./100 ml.		Diffusible calcium, mg./100 ml.		Non-diffusible calcium, mg./100 ml.	
	No. birds	Mean ±S.E.M.	No. birds	Mean ±S.E.M.	No. birds	Mean ±S.E.M.
Normal	73	24.7±0.6*	73	7.0±0.2*	73	17.1±0.6*
18 to 24-hour Starvation	20	16.5±0.9	6	5.9±0.1†	6	9.2±1.4
Sham Parathyroidectomy	10	16.4±0.7	10	5.7±0.2‡	10	10.7±0.7
Parathyroidectomy	27	11.3±0.6*	27	4.3±0.3*	27	7.3±0.5*

* $P < .01$ Comparison to sham operated group.
† The mean diffusible calcium level of these hens immediately before starvation was 5.5 ± 0.3 mg./100 ml.
‡ 7 of 10 hens had hard shell eggs in uterus when blood sample was removed.

TABLE 4. THE EFFECT OF SHAM PARATHYROIDECTOMY AND PARATHYROIDECTOMY ON MORTALITY, SHELL DEPOSITION AND EGG PRODUCTION

Sham parathyroidectomy	Parathyroidectomy
Mortality	
0 of 10 died	8 of 30 died a) 3 during 1st night b) 5 within 4 days
Shell Deposition	
8 of 10 due to lay eggs a) 7 laid hard shell eggs b) 1 laid egg prematurely with no shell	21 of 27 due to lay eggs a) 2 laid hard shell eggs b) 10 laid eggs prematurely with some shell c) 9 laid eggs prematurely with no shell

normal diffusible calcium levels, and showed no signs of hypoparathyroidism. Most of the sham operated hens did not eat until the morning after the operation, probably because of surgical trauma. Thus, effects of starvation must be considered in an evaluation of the data obtained on all operated hens.

Soon after the extirpation of the parathyroids, eggs present in the uterus were prematurely expelled with little or no evidence of shell deposition. In 19 of 21 cases, the eggs were laid about 3–7 hours after the operation (Table 4). The other 2 parathyroidectomized hens laid their eggs on schedule with a full complement of shell calcium. These two hens at the time of oviposition had plasma calcium levels of 19.7 and 13.3 mg./100 ml. Two days later, the hen with the higher calcium level exhibited signs of hypoparathyroidism and died. The other hen resumed egg production 3 days after the operation. Sham operated hens laid their eggs on schedule and the shell weights were comparable to non-operated controls, 4.87 and 5.05 gr., respectively. Thus sham operation had no effect on shell deposition other than that resulting from handling and possible effects from starvation.

The number of days to first shell egg (6 days) was similar for both 48 hour starved or parathyroidectomized hens (Table 5), suggesting that starvation may partially account for the interference in egg production. A few of the operated hens did not resume production for 16 days, and some for as long as 32 days. Sham operated hens, on the other hand continue to lay eggs.

TABLE 5. THE EFFECT OF 48-HOUR STARVATION, SHAM PARATHYROIDECTOMY AND PARATHYROIDECTOMY ON SUBSEQUENT EGG PRODUCTION OF LAYING HENS

	No. of days to first shell egg after experimental regimen*		
	48 hour starvation	Parathyroidectomy	Sham parathyroidectomy
No. of Birds	10	11	10
Mean ±S.E.M.	5.5±1.3	5.9±1.7	1.6±0.2

* Does not include 1st day after operation.

After the parathyroidectomized hens resume production, eggs are laid of weight similar to those laid before the operation (Table 6). Shell weight and per cent shell weight were also similar to pre-operation values. Nonidez and Goodale (14) reported that hens, which recovered from parathyroidectomy, had accessory tissue that underwent hyperplasia and hypertrophy. Other investigators (10, 15, 16, 17) had shown that accessory parathyroid tissue was prevalent in chickens. Presumably the presence of this accessory tissue in laying hens could account for their recovery from the operation and their return to egg production.

Discussion. The data show: 1) that shell deposition caused a decrease in the diffusible plasma calcium, 2) that the diffusible calcium level can fluctuate within the normal, intact hen, and 3) that ablation of the parathyroids caused a marked decrease in both diffusible and non-diffusible plasma calcium, and when performed at the onset of shell deposition resulted in premature expulsion of the egg. Thus, it appears that shell deposition is dependent upon the maintenance of the diffusible calcium level in the blood and that the parathyroid glands are involved in the maintenance of this level; thereby exerting an indirect influence on shell deposition.

TABLE 6. DATA ON EGG WEIGHT AND SHELL WEIGHT OF EGGS LAID BY HENS BEFORE AND AFTER PARATHYROIDECTOMY

No. of birds:	Egg weight, gm.		Shell weight, gm.		% shell weight	
	Control*	Exptl.†	Control	Exptl.	Control	Exptl.
	7	7	7	7	7	7
Mean	58.45	59.38	4.80	4.43	8.22	7.48
S.E.M.	±1.08		±0.25		±0.50	
P	>.05		>.05		>.05	

* = 10 day control period before operation.
† = 16 day experimental period after operation.

The significant decrease in the plasma diffusible calcium level caused by shell deposition in the intact hen is interpreted as indicating that the rate of calcium deposition on the shell may, at times, exceed the rate of calcium mobilization. When this occurs, the lowered diffusible calcium concentration provides the stimulus for an increased mobilization of calcium. This could induce the movement of labile bone calcium into the vascular system (18) or the elaboration of the parathyroid hormone, which, in turn, brings about bone calcium mobilization or a possible rise in the kidney threshold for ionic calcium (19). Investigators have shown that a decreased level of blood calcium resulted in an increased parathyroid activity in mammals (20, 21, 22) and that a relationship existed between parathyroid and bone histology during shell deposition in birds (5). Thus, there is some basis for a suggestion that the alternating level of diffusible calcium in the hen influences the output of parathyroid hormone, which,

by its calcium mobilizing ability, in turn influences the level of the diffusible calcium. The significant decrease in the diffusible calcium level following extirpation of the parathyroids from laying hens adds further support to this theory. Also, parathyroid extracts injected into parathyroidectomized birds causes a remission of symptoms and increases blood calcium in these (unpublished) and normal birds (7).

The decrease in the non-diffusible calcium that occurred in parathyroidectomized hens, is not attributed to the removal of the parathyroids, *per se*. Starvation accounted for much of the decrease of this fraction, and an estrogenic imbalance or decreased end-organ response presumably accounted for the remainder of the decrease. Later studies, which will be subsequently published, show that estrogen does not increase the non-diffusible calcium when the diffusible calcium level is below normal in parathyroidectomized male and gonadectomized male chickens. This is in agreement with the results obtained with laying hens, in this study, in which non-diffusible calcium was not maintained by endogenous estrogen in hens that had a below-normal diffusible calcium level after parathyroidectomy.

Thus, in the hen, as in the pigeon (3) the decrease in total calcium following parathyroidectomy is primarily attributed to the decrease in the diffusible calcium fraction.

As removal of the parathyroids from the hen resulted in a marked decrease in the plasma calcium fractions, a more rapid loss of blood calcium would be expected if shell deposition were occurring in the parathyroidectomized hen. Such hens were observed to expel their eggs prematurely, about 3–5 hours after parathyroidectomy. Symptoms of hypoparathyroidism did not occur in as short a time as this in hens parathyroidectomized but not depositing shell calcium. Presumably, diffusible calcium decreased to abnormal levels, particularly at the uterine level where shell deposition was occurring. As a result, an ion imbalance caused hyper-irritability (Stewart and Percival, 1928) of the uterine musculature with expulsion of the egg. If the egg remained (as observed in 3 hens found dead the morning after the parathyroidectomy) then the diffusible calcium decreased to a level at which death occurred. If the egg were expelled from the uterus, as observed in 19 of 21 hens, the stimulus for shell deposition was removed and the hen had a chance for survival.

SUMMARY

Laying hens show a marked decrease in diffusible and non-diffusible plasma calcium after parathyroidectomy, and prematurely expel uterine eggs about 3–5 hours after the operation. In addition, shell deposition significantly influences diffusible calcium levels in the normal laying hen. These data are discussed in relationship to the role the parathyroids play in regulating plasma calcium concentration and thus, indirectly influence shell deposition.

REFERENCES

1. Benoit, J., G. Fabiani, R. Grangaud and J. Clavert: *C. R. Soc. Biol.* **135:** 1606. 1941.
2. Benoit, J.: *Traité de Zool.* ed. P. P. Grasse, Tome XV, Oiseaux. Masson and Co., Paris. 1950.
3. Riddle, O. and M. R. McDonald: *Endocrinology* **36:** 48. 1945.
4. McDonald, M. R. and O. Riddle: *J. Biol. Chem.* **159:** 445. 1945.
5. Bloom, W., M. A. Bloom and F. C. McLean: *Anat. Rec.* **81:** 445. 1941.
6. Sun, T. P. and M. M. MacOwen: *J. Physiol.* **70:** IV. 1930.
7. Polin, D. and P. D. Sturkie: *Endocrinology.* **60:** 1. 1957.
8. Moritz, A. R.: *J. Biol. Chem.* **66:** 343. 1925.
9. Benoit, J., P. Stricker and G. Fabiani: *C. R. Soc. Biol.* **135:** 1600. 1941.
10. Higgins, C. W. and C. Sheard: *Am. J. Physiol.* **85:** 299. 1928.
11. Campbell, I. L. and C. W. Turner: *Univ. Mo. Res. Bull.* 352. 1942.
12. Comar, C. L. and J. C. Driggers: *Science* **109:** 282. 1949.
13. Driggers, J. C. and C. L. Comar: *Poult. Sci.* **28:** 420. 1949.
14. Nonidez, J. F. and H. D. Goodale: *Am. J. Anat.* **38:** 319. 1927.
15. Bomskov, C.: *Methodik der Hormon forschung* **1:** 142. 1937.
16. Bargmann—see Benoit, J. (2).
17. Dudley, J.: *Am. J. Anat.* **71:** 65. 1942.
18. McLean, F. C. and M. R. Urist: *Bone.* Univ. of Chicago Press, Chicago, Ill. 1955.
19. Talmage, R. V.: *Ann. N. Y. Acad. Sci.* **64:** 326. 1956.
20. Ham, A. W., N. Littner, T. G. H. Drake, E. C. Robertson and F. F. Tisdall: *Am. J. Pathol.* **16:** 277. 1940.
21. Patt, H. M. and A. B. Luckhardt: *Endocrinology* **31:** 384. 1942.
22. Stewart, G. and H. E. Bowen: *Rev. Canad. Biol.* **10:** 88. 1951.
23. Stewart, C. P. and G. H. Percival: *Physiol. Rev.* **8:** 283. 1928.

THE RETARDING INFLUENCES OF ADRENALECTOMY AND AGE ON ESTRADIOL-INDUCED CONNECTIVE TISSUE HYPERPLASIA IN SEMINAL VESICLES[1]

EVELYN HOWARD AND J. C. ALLEN

Department of Physiology, Johns Hopkins University, School of Medicine, Baltimore, Maryland

ESTROGENS are known to stimulate hyperplasia of the fibro-muscular walls of certain portions of the male reproductive tract. In young castrated rats treated with estrogen, hyperplasia of fibro-muscular tissue in the seminal vesicles has been described by Freud (1), and comparable changes in mice seminal vesicles were noted by de Jongh (2). Korenchevsky and Dennison (3) described the histological effects of estrogen in more detail, and emphasized specifically the increase in the fibrous tissue of the submucosa in the seminal vesicles of castrated rats. In young estrogenized monkeys, Van Wagenen (4) found the most striking size increase to occur in the seminal vesicles, the increase in weight consisting of a fibro-muscular hyperplasia. However, Lacassagne (5) noted atrophic seminal vesicles in estrogenized mice in the presence of marked epidermoid hyperplasia of the coagulating gland, so that the factors influencing the action of estrogens on the fibrous tissue of the seminal vesicles do not seem to be completely defined.

Cortisone interference with the growth of connective tissue has been described by various authors, cited by Meier *et al.* (6), and the formation of new connective tissue around a subcutaneous cotton pellet has been shown to be depressed by cortisone to a degree correlated with the generalized inhibition of body growth by the steroid (6). Some of the well known effects of cortisone at relatively high dosage, such as growth inhibition (Ingle and Prestrud, 7), diabetogenic effect (Houssay *et al.*, 8), and lowered resistance to infection (Robinson *et al.*, 9), appear to be opposite to the effects of normal adrenal function, or the effects of small doses of corticoids. In view of the apparent bimodality of these corticoid effects, it seemed reasonable to inquire whether the adrenals do not promote the development of connective tissue, at least under some circumstances. As a

Received December 28, 1956.

[1] Desoxycorticosterone acetate and estradiol dipropionate were generously furnished by Ciba Pharmaceutical Products, through the courtesy of Dr. Robert Gaunt. The work was supported in part by U.S.P.H.S. grant #H2414. A preliminary report on part of this work was presented before the American Association of Anatomists Annual Meeting, 1955. Preparation of tissues for histological study was done by Miss Cecilia Bisson, Miss Catherine Howard, and Mrs. Doris Goode.

favorable type of connective tissue response for study, we chose the connective tissue hyperplasia induced by estrogen in the seminal vesicles, a further consideration being that the adrenals might be expected to have some relationship to events in the reproductive system. That the adrenals do indeed promote the development of this connective tissue is indicated by the observations to be presented, which show that the estradiol stimulation of connective tissue formation in the seminal vesicles of young castrated mice is reduced if the animals are adrenalectomized.

EXPERIMENTAL

Mice of the CFW[2] strain were operated on at two ages, namely at 19 to 21 days and at 8 to 9 weeks. The older group showed little or none of the effects being discussed. The younger group will be described first. One member of a litter mate pair was castrated, the other one castrated and adrenalectomized at one sitting. At the time of operation, pellets of desoxycorticosterone acetate (DCA) were implanted at the adrenal site. These usually became embedded in liver tissue, which was desirable in order to minimize the amount of the steroid in the body fluids. The castrated animals were usually given DCA pellets also, but in some cases DCA was omitted in the castrates without detectable effects on the vesicle reaction being studied. Alpha-estradiol dipropionate pellets[3] were implanted subcutaneously in all except a few untreated blanks. The mice were returned to their mothers 4 to 6 hours after operation and allowed to nurse, usually until they were four weeks of age. They survived the operation well, and the two groups grew at approximately identical rates, the initial mean body weight being 10.1 gm. for both groups; and at autopsy the adrenalectomized castrates had gained 13.2 gm. (mean) and the castrates 13.3 gm. They were autopsied at 20 or 40 days after operation, both members of a pair being autopsied on the same day. The hypertrophy induced by the estradiol was not markedly accentuated by the longer period of observation. The estradiol dipropionate was absorbed at the rate of 0.02 to 0.03 mg. per day, the DCA at 0.14 mg. per day.

At autopsy the vesicles were immersed in Ringer's saline and dissected free of the coagulating gland and other attachments under a dissecting microscope. They were then fixed in Bouin's fluid, and on the following day weighed to 0.02 mg., after being immersed in water for several hours, and drained briefly on filter paper in a standard manner. Thereafter, vesicles were sectioned at 6 to 10 micra, and stained either in Azan or in Van Gieson's picric acid-acid fuchsin (10).

The figures listed in the Table show, in the case of the groups not treated with estradiol, no significant differences in the weights of the vesicles in the adrenalectomized castrates compared to litter mate castrates, confirming previous findings (11), on larger numbers of animals. Similarly, treatment of a small group with pellets of 1% estradiol dipropionate in cholesterol revealed no evidence of any supportive effect of the adrenal. However, in the groups of animals treated with pellets of 100% estradiol

---

[2] Carworth Farms.

[3] In some later observations on another strain of mice, 100% estradiol pellets were unduly toxic to adrenalectomized mice. The use of pellets 50% estradiol in cholesterol gave satisfactory survival of the adrenalectomized and induced connective tissue hyperplasia in the presence of the adrenal.

TABLE 1. EFFECT OF ADRENALECTOMY ON THE ESTRADIOL INDUCED HYPERTROPHY OF SEMINAL VESICLES IN YOUNG MICE

Treatment		No. of mice	Vesicle wt., mg. / Body wt., gm.	P
Operation	Estradiol pellet			
AC	none	4	7.82	—
C	none	3	8.04	
AC	1%	4	7.18	—
C	1%	3	5.27	
AC	100%	17	7.02	0.01
C	100%	20	15.96	

Explanation: A, Adrenalectomy, C, castration, P, probability of chance distribution of observed values.

dipropionate, it appears that castrated animals with their adrenals intact have vesicles which show a mean weight twice that of the adrenalectomized castrates, a difference with a probability of occurrence by chance of less than one per cent. There was, however, considerable variability in the vesicle reaction in the castrates, further discussed below. This variation in the vesicle weights was associated with marked differences in histological structure.

The reactions of mice seminal vesicles to castration at various ages have previously been described (12). At 21 days-of-age, the vesicles are made up of a fairly complicated structure of acini lined with columnar but not fully mature epithelium. Castration at this age is followed by much less regressive change in seminal vesicles than follows castration of adults: the epithelium and the general structure may show no change for several weeks. Furthermore, adrenalectomy was not followed by an appreciable change in the histological appearance of the vesicles of castrates when the animals were maintained in good condition by means of desoxycorticoterone (11).

In the present study, the adrenalectomized castrates treated with estradiol pellets showed in most cases no hypertrophy of connective tissue. Thus hypertrophy of connective tissue was not seen in fifteen of twenty adrenalectomized, estrogenized mice, while three showed slight thickening of the wall, and two showed moderate thickening. (One of the latter had an accessory nodule of enlarged cortical cells. In the other cases, accessory nodules were not noted on examination of the adrenal sites, under 15×magnification, but their presence was not excluded). While the vesicle wall usually remained thin, the general architecture of the acini was unchanged. The condition of the epithelium was rather variable: it was not always maintained in its columnar state, but was often cuboidal, and sometimes desquamating or showing degenerative vacuolization. Estrogen damage to rat seminal vesicle epithelium in castrated rats has been described by David, Freud and de Jongh (13).

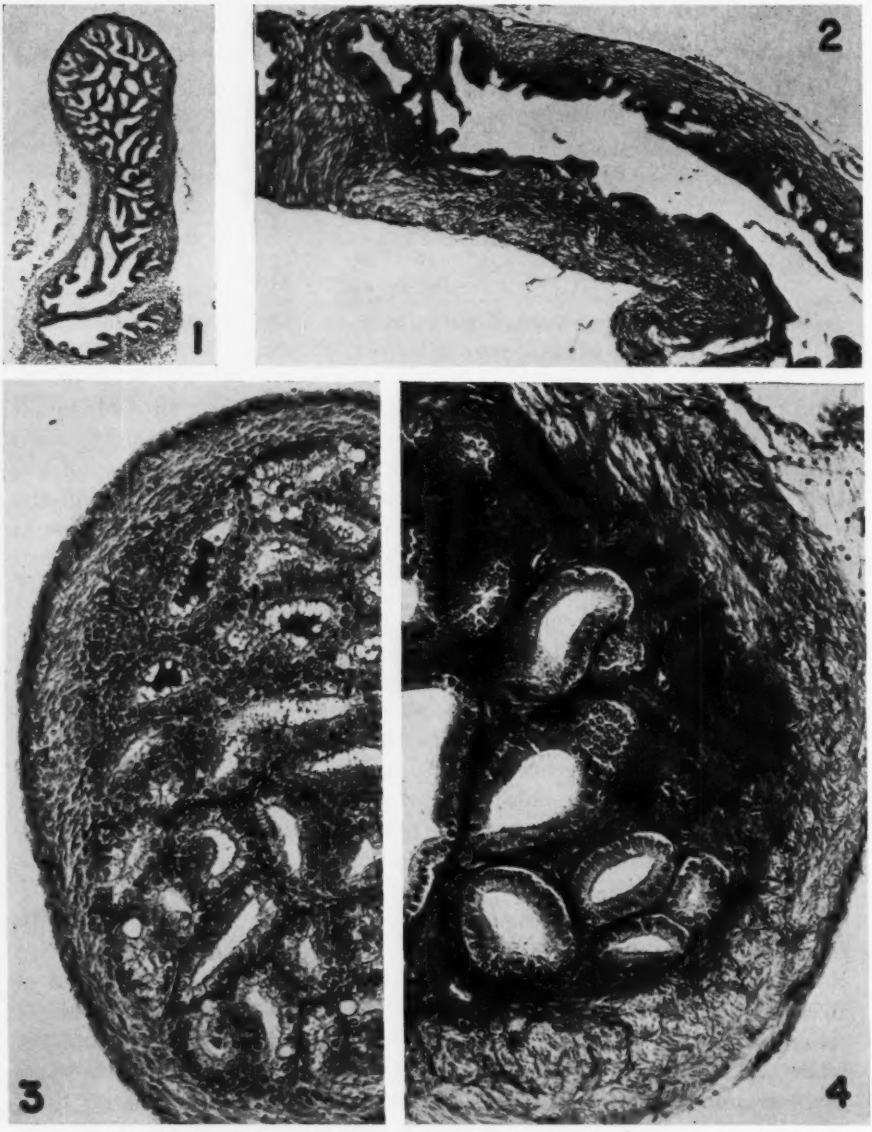


FIG. 1. Seminal vesicle of an adrenalectomized castrated mouse, operated on at 21 days-of-age, implanted subcutaneously with a pellet of estradiol dipropionate, and autopsied after an interval of 38 days. Note the complex acinar structure characteristic of the secretory portion of the vesicle, and that the structure becomes less complex as the collecting ducts are approached. This appearance is also typical of that of the untreated castrate or untreated adrenalectomized castrate. There are narrow blue basement membrane components, but most of the wall structure, as well as the epithelium, stains pink in azan. ×50.

The vesicles of the estrogenized castrates with intact adrenals were divided into groups according to the degree to which they exhibited connective tissue hyperplasia with regression of acinar development, the groups being arranged on the basis of the histological structure without regard to the weights. The changes are illustrated in the photographs. Of 23 animals studied histologically, 5 showed no change and 4 showed only slight hyperplasia. The mean weights of these vesicles did not differ significantly from the mean weights of the 17 adrenalectomized castrates, 7.0 vesicle-body weight ratio. Significant enlargement occurred in those vesicles with histological evidence of moderate hyperplasia, 10 animals with a mean vesicle-body weight ratio of 12.7 ($P < 0.01$) and in those showing advanced hyperplasia, four animals with a mean vesicle-body weight ratio of 36.3 ($P < 0.001$). The response to estrogenization thus appeared to be highly variable, whether judged by vesicle weights or by histological state. The reason for this great variability is not clear. An unexpected observation was that animals which showed little or no vesicle hyperplasia had adrenal X zones which showed little or no degeneration, i.e., the adrenals were in a state which would be expected in untreated castrates of this age (12), whereas animals with moderate and advanced vesicle hyperplasia had X zones which were frequently in a state of advanced degenerative vacuolization, or essentially without remaining X zone cells.

The thickening of the wall of the vesicle in the estrogenized young castrated mice is associated with an increase in the components which

FIG. 2. Seminal vesicle of a castrated mouse on estradiol, treated similarly to the mouse shown in Figure 1, except that the mouse of Figure 2 had intact adrenals. Note the simple structure which was characteristic of the entire vesicle. Except for the lining epithelium and the nuclei, the wall is made up of connective tissue which stains an intense blue in azan. This vesicle was classified as showing advanced hyperplasia. The developing connective tissue has entirely replaced the smooth muscle of the wall, and the complex structure shown in Figure 1 has been converted into a thick walled tube with only a few remaining outpocketings. ×50.

FIG. 3. Higher magnification of a vesicle of an adrenalectomized, castrated mouse, which had been treated with estradiol for 38 days. This structure does not differ appreciably from that of an untreated castrated mouse or an untreated adrenalectomized castrate. Note the moderately well developed columnar or cuboidal epithelium, and thin basement membranes of the acini. As in Figure 1, except for very thin basement membrane components, the wall stains predominantly the pink characteristic of smooth muscle in the azan stain. Some acini contain small amounts of dark staining secretion. ×200.

FIG. 4. The vesicle of a castrated mouse which had carried an estradiol pellet for 38 days, a litter mate of the mouse shown in Figure 3, and identically treated except that the adrenals were intact. Note the invasion of the blue staining connective tissue, printed dark, between and around the acini. The outer part of the wall, the lighter area, is made up, predominantly, of pink staining elements, but the dark area is entirely made up of the hyperplastic connective tissue, staining an intense blue in azan. In this case the acini are fairly intact. This specimen was classified as showing moderate hyperplasia, the changes not having progressed as far as in the vesicle shown in Figure 2. ×200.

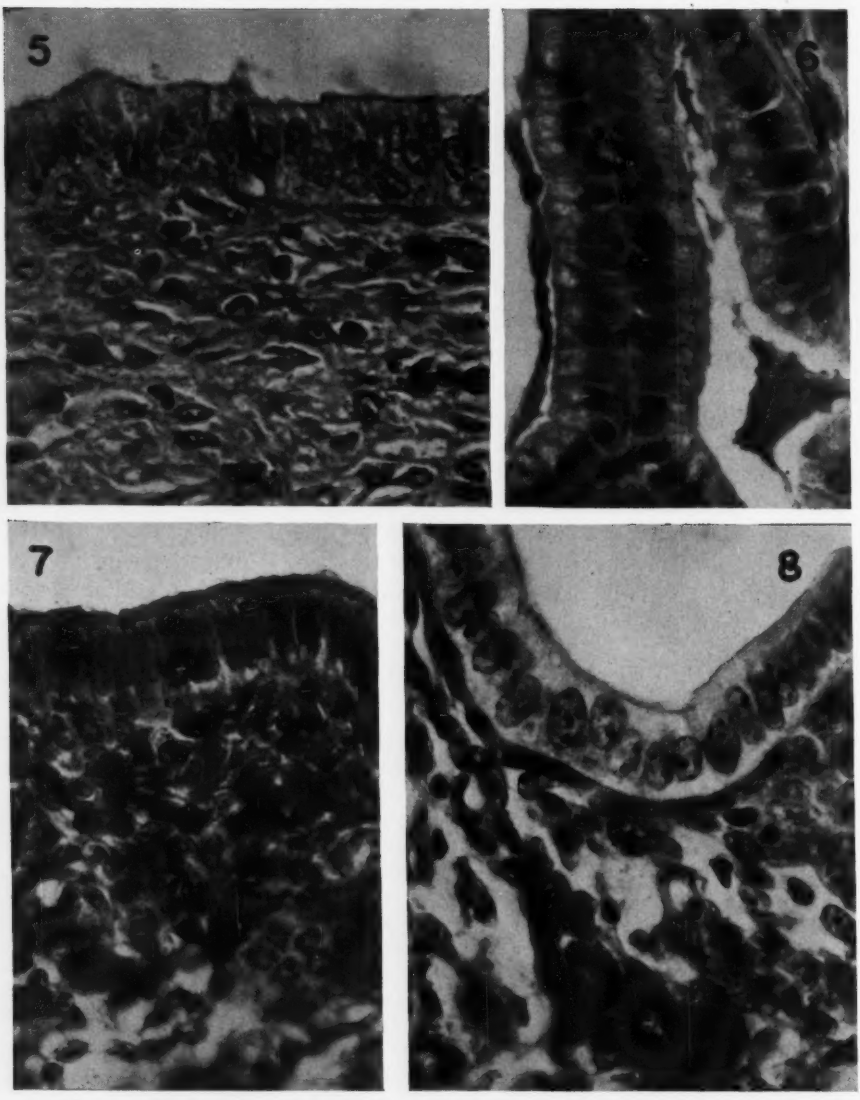


FIG. 5. Epithelium typical of maximum seminal vesicle response to estrogen, from the lining of a vesicle from a mouse which had been castrated at 21 days-of-age, and treated with an estradiol pellet (50% in cholesterol) for 19 days. Fibrous hyperplasia had produced a vesicle structure similar to that shown in Figure 2. Note that the epithelium is composed of cells which are cylindrical and that the nuclei tend to be elongated, and centrally placed in contrast to the nuclei of the normal intact or untreated castrate vesicle, which are basal and more nearly spherical while the cells are high columnar in the intact, and cuboidal or low columnar in the untreated castrate. In shape and position the nuclei of the estrogenized vesicle thus tend to resemble the nuclei of uterine epi-

stain selectively blue in the azan technique or pink in the Van Gieson's, i.e., staining reactions which are characteristic of collagenous and elastic connective tissue fibres (10). In the adrenalectomized group, the material staining blue in azan was confined to the very thin surface membrane and the sometimes visible very thin components of the basement membrane of the epithelium. The vesicles which hypertrophied, in the animals with their adrenals, showed a pronounced thickening of the basement membrane blue staining material, which was interspersed between the acini when the hyperplasia was only moderate (Fig. 4). This degree of change resembles the reaction in castrated estrogenized vesicles, described by Korenchevsky and Dennison (3). With advanced hyperplasia, however, the acini in the present material became almost entirely replaced by blue staining connective tissue, so that the vesicle consisted of a simple tube with minor outpocketings, lined with narrow cells arranged with their long axes at right angles to the epithelial surface, as illustrated in Figures 2 and 5. This epithelium bears a certain resemblance to uterine epithelium, shown for comparison in Figures 7 and 8. Not only does the blue staining connective tissue replace the complex acinar structure, but it also replaces the muscular tissue of the vesicle wall. Normal mice, as well as the adrenalectomized castrates, have vesicle walls which contain a considerable amount of smooth muscle, staining pink or lavender in azan. In moderate hyperplasia, illustrated in Figure 4, the blue staining thickening of the basement membrane is conspicuous, but it is surrounded by an area staining similarly to smooth muscle. However, in the cases of advanced hyperplasia, (Figures 2 and 5) the entire wall of most of the vesicle, except for the lining epithelium, was made up of blue staining material with an abundance of red nuclei.

---

thelium rather than normal vesicle epithelium. Note that there is no tendency of the vesicle epithelium to stratify, although the prostatic coagulating gland of this specimen showed marked squamous metaplasia of the epithelium. This vesicle produced no secretion. Azan. ×750.

FIG. 6. Intact male seminal vesicle epithelium, from a mouse 61 days-of-age. This epithelium was producing abundant secretion. Note that the actual cell height does not exceed that of the estrogenized castrate, but that the position of the nucleus is quite different. Secretory granules and characteristic "light areas" are present in the cytoplasm. Castration without other treatment in adult males reduces the height of the epithelium, as illustrated in detail by Howard (12). Haematoxylin. ×750.

FIG. 7. Epithelium from the uterus of a mouse which had been ovariectomized at 12 days-of-age and autopsied after an interval of 19 days. Note crowded epithelial cells with elongated nuclei. Azan. ×750.

FIG. 8. Uterine epithelium from normal female 42 days-of-age, with well developed corpora lutea present in the ovaries. Note that the cells are larger and hence the nuclei less crowded than in the ovariectomized, but that the nuclei are oval with some resemblance to those of the estrogenized vesicle, and that the ratio of cell height to nuclear length is similar to that of the estrogenized vesicle. The stroma is, however, quite different. Azan. ×750.

In those individuals which exhibited lesser degrees of connective tissue hyperplasia, the vesicle epithelium showed less change from the condition characteristic of the untreated castrates: i.e., the vesicle epithelium was generally rather low cuboidal, with small areas in which the tendency toward columnar cells persisted. The possibility that estrogens initially tend to hasten the reduction of the normally columnar epithelium to the cuboidal state (13) could neither be confirmed nor excluded on the basis of the present material. However, in vesicles exhibiting the greater degrees of connective tissue hyperplasia, the epithelium showed a characteristic alteration to the condition illustrated in Figure 5, in which the cuboidal cells have been changed into close-packed cylindrical cells with elongated centrally placed nuclei bearing some resemblance to the cells of uterine epithelium. No stratification of vesicle epithelium ever occurred even in those specimens showing the most marked fibrous tissue development.

In the coagulating gland, on the other hand, replacement of the columnar epithelium by stratified epithelium was apparently complete. This was noted in all the coagulating glands examined in the younger group, namely 4 castrates on estradiol and DCA, 4 adrenalectomized castrates on estradiol and DCA, and 5 adrenalectomized castrates on estradiol, DCA and corticosterone. The degree of cornification varied considerably, and, while quantitative comparisons were not made, the material suggests that corticosterone may hasten the estradiol-stimulated invasion and cornification of the stratified epithelium. It may be concluded that the seminal vesicle is much less subject to the invasion of stratified epithelium than is the adjacent coagulating gland under the conditions of this study.

In the group of mice in which the operations were performed at the somewhat older stage of 8 to 9 weeks-of-age, after the primary X zone had involuted, nine pairs of mice were operated on and given pellets of estradiol and DCA as described above for the younger ones. They were autopsied after three weeks. The vesicle body weight ratios in the castrated mice were not found to differ from those in the adrenalectomized castrates, nor did they differ from the values in non-estrogenized castrates. Histologically the vesicles of these older mice were in a remarkably uniform state, in both the adrenalectomized castrates and the plain castrates. The epithelium had, for the most part, regressed to a cuboidal condition. The vesicle wall was moderately thick. The basement membrane beneath the epithelium was perhaps slightly thickened, in comparison to the intact adult vesicle, but the acinar structure had in no case been replaced by connective tissue. The wall itself was primarily composed of elements staining the pink or lavender characteristic of smooth muscle, usually with little admixture of blue elements. There were more blue elements in these vesicles than were present in the actively secreting vesicles of intact males at 6 weeks-of-age, but not more than was normal for intact males 6 months-of-age. There were no differences related to the presence or absence of the

adrenal in these mice. In this group three weeks after castration, moderate sized secondary X zones were present. In two of these older castrated mice the coagulating gland was examined, and found to show no sign of the invasion of stratified epithelium, suggesting that there is a marked age difference in the resistance to estradiol-induced alteration in this gland, comparable to that of the seminal vesicles.

The effect of corticosterone on the fibrous hyperplasia was studied in a group of 5 mice castrated and adrenalectomized at 21 days-of-age and given, in addition to pellets of DCA and estradiol, intraperitoneal pellets of 40% corticosterone in cholesterol. These pellets yielded corticosterone at the rate of 0.02 mg. per day; a dose compatible with nearly normal body growth. It was found that in all mice so treated there was a distinct hyperplasia of the blue staining connective tissue elements, producing a situation similar to that seen in the presence of the adrenal. Although quantitative comparisons were not made, it may be concluded that replacement of the adrenal by DCA plus corticosterone is compatible with connective tissue hyperplasia in the estrogenized vesicle. In one case corticosterone was tested without estradiol, and no connective tissue hyperplasia occurred.

COMMENT

A relatively rapid connective tissue hyperplasia is characteristic of the seminal vesicles of immature castrated mice under the influence of estradiol, whereas young adult castrated mice failed to show marked changes under the same conditions. The difference in reactivity suggests a greater lability of the younger vesicles. It is of interest that the fibrosis is associated with actual enlargement of the vesicle, and therefore cannot be simply a relative fibrosis due to loss of parenchyma, but there must be a positive stimulus to the formation of fibrous connective tissue. The presence of the intact adrenal X zone (25) was not necessary for the fibrous reaction to occur, in fact, the most extreme changes occurred in individuals in which the X zone was undergoing concurrent degeneration. However, the series is too small to conclude with assurance that the presence of the intact X zone tends to protect against the estradiol effect.

The presence of the adrenal itself exerted a favorable influence on the estrogen induction of connective tissue hyperplasia in the vesicles of the immature castrated mice, and corticosterone with DCA was able to substitute for the adrenal in this situation. General body growth in the adrenalectomized castrates maintained on DCA and estradiol proceeded at a normal rate in the present series, body weight having been more than doubled during the experimental period. In contrast, the cortisone-induced reduction in connective tissue growth around a subcutaneous cotton pellet, which was described by Meier *et al.* (6), was correlated with generalized inhibition of body growth by the steroid. The present observations indicate that, although excessive corticoids may interfere with the prolifera-

tion of connective tissue, nevertheless adrenal hormones, such as corticosterone, appear to exert a favorable effect, at some level, on the processes of estradiol-induced connective tissue hyperplasia. Estrogens are known to stimuate cortical activity (14). The present observations suggest a synergism between estrogen and corticoids in the induction of a rapid connective tissue hyperplasia in the seminal vesicles of immature castrated mice.

The alterations in the young vesicle structure under the influence of estradiol produce, in the more extreme cases, an organ which has a certain resemblance to uterus, inasmuch as the complicated acinar structure is replaced by a relatively simple tube-like structure with a thick wall, and the epithelium changes to narrow cylindrical cells which have a resemblance to the epithelial lining of the uterus, being somewhat intermediate between the ovariectomized and the postovulatory uterus, as shown in Figures 7 and 8. The resulting structure differs from uterus in that the stroma contains only blue staining connective tissue and lacks the muscular elements, hence the change is at most only a change in some respects toward the characteristics of uterus. In view of the evidence for critical periods in development which limit genital tract reactivity to the heterotypic hormone, discussed by Burns (15), one would expect that any tendency of a male structure to change toward a female structure would decline with increasing age. This expectation was fulfilled in the present instance, in that the rapid connective tissue hyperplasia under the influence of estradiol was characteristic of the seminal vesicles of mice when treatment was begun at 3 weeks-of-age, but not at 7 to 9 weeks. It is possible that the variations in the degree of hyperplasia within the younger group depend on variations in the initial degree of vesicle differentiation which is present at the initiation of treatment. One would expect that the maximal changes from the normal status of the mature vesicle would be produced if one started with a fairly simple structure with a high degree of connective tissue responsiveness of estradiol. The fact that a high degree of connective tissue responsiveness appears to be limited by ontogenetic factors suggests the possibility that the connective tissue hyperplasia produced by long term treatment with estrogens as noted by various authors (16, 17) might be associated with a reversion of connective tissue metabolic properties to a less differentiated status than that characteristic of the normal adult.

The susceptibility of younger vesicles to connective tissue invasion finds a parallel in the greater susceptibility of young rats to the development of hypertension and pathological vascular alterations following adrenal enucleation under the conditions employed by Skelton *et al.* (19). Differences in the amount of growth hormone furnished by the pituitary may be a factor in the two age groups. A stimulating effect of growth hormone on the proliferation of granulation tissue around tupentine abcesses has been noted by Taubenhaus (20), who found growth hormone to be ineffective in adrenalectomized animals, suggesting a parallel to the present observa-

tions. A synergism between growth hormone and cortisone in promoting the growth of fibroblasts in tissue culture has been noted (Leslie, 21).

An effect of the adrenal on specific cellular contact forces might also be a factor in the connective tissue invasion of the vesicle. Cortisone has been found to promote metastasis (22, 23, 24), and to increase the receptivity of a variety of tissues to tumor cells after inoculation into the left cardiac ventricle (24). It seems possible that some effect on tissue receptivity or cellular contact forces is associated with the favorable effect of the adrenal on fibrous tissue growth in the immature seminal vesicles in the present experiments. In any case, in the estrogenized vesicles some favorable effect on receptivity to fibrous tissue evidently dominates the situation in preference to the inhibitory effect of corticoids on fibrous tissue proliferation, manifest at high dose levels in other experimental situations. It might be expected that immature tissue would be more susceptible to alteration by fibrous hyperplasia because of incomplete development of specific properties including specific intercellular binding groups, hence it is logically satisfying to find that the mature vesicles are not susceptible to fibrous hyperplasia under the conditions employed.

SUMMARY

The presence of the adrenal promotes the induction of a rapidly developing connective tissue hyperplasia in the seminal vesicles of immature mice treated with estradiol. Older mice are much more resistant to this effect of estradiol.

This reactivity to estradiol is interpreted as a response of cellular elements which in three-week-old mice are not irreversibly differentiated in a male direction, but which by eight to nine weeks-of-age have lost this degree of reactivity to the heterologous sex hormone.

The effect of the adrenal may be related to a loosening of specific intercellular bondings creating a more favorable soil for fibroblast proliferation.

REFERENCES

1. Freud, J.: *Biochem. J.* **27**: 1438. 1933.
2. de Jongh, S. E.: *Arch. Internat. de Pharmac. et de Therapie.* **50**: 348. 1935.
3. Korenchevsky, V. and M. Dennison: *J. Path. and Bacter.*: **41**: 323. 1935.
4. Van Wagenen, G.: *Anat. Rec.* **63**: 387. 1935.
5. Lacassagne, A.: *Compt. Rend. des Seances de la Soc. de Biol.* **113**: 590. 1933.
6. Meier, R., W. Schuler and P. Desaulles. *Experientia* **6**: 469. 1950.
7. Ingle, D. J. and M. C. Prestrud: *Endocrinology* **45**: 143. 1949.
8. Houssay, B. A., R. R. Rodriguez and A. F. Cardeza: *Endocrinology* **54**: 550. 1954.
9. Robinson, H. J., R. C. Mason and A. L. Smith: *Proc. Soc. Exper. Biol. and Med.* **84**: 312. 1953.
10. Lillie, R. D.: *Histopathological Technique.* Philadelphia, 1954.
11. Howard, Evelyn: *Endocrinology* **38**: 156. 1946.
12. Howard, Evelyn: *Am. J. Anat.* **65**: 105. 1939.
13. David, K., J. Freud and S. E. deJongh: *Biochem. J.* **28**: 1360. 1934.

14. TEPPERMAN, J., F. L. ENGEL AND C. N. H. LONG: *Endocrinology* **32**: 373. 1943.
15. BURNS, R. K.: *Biol. Symposia* **9**: 125. 1942.
16. GARDNER, W. U. *Surgery* **16**: 8. 1944.
17. LIPSCHUTZ, A. *Steroid Hormones and Tumours*. Baltimore, 1950.
18. KIRKMAN, H. AND R. L. BACON. *J. Nat. Cancer Institute* **13**: 757. 1952.
19. SKELTON, F. R. AND J. GUILLEBEAU: *Endocrinology* **59**: 201. 1956.
20. TAUBENHAUS, M. AND G. D. AMROMIN: *J. Lab. and Clin. Med.* **36**: 7. 1950.
21. LESLIE, I.: *Biochem. J.* **52**: xxi, 1952.
22. AGOSIN, M., R. CHRISTEN, O. BADINEZ, G. GASIC, A. NEGHME, O. PIZARRO AND A. JARPA: *Proc. Soc. Exp. Biol. and Med.* **80**: 128. 1952.
23. BASERGA, R. AND P. SHUBIK: *Cancer Research* **14**: 12. 1954.
24. POMEROY, T. C.: *Cancer Research* **14**: 201. 1954.
25. HOWARD, EVELYN: *Am. J. Anat.* **40**: 251. 1927.

PEPSIN INHIBITOR IN PITUITARY EXTRACTS

JESSAMINE HILLIARD AND PHILIP M. WEST[1]

Departments of Anatomy and Biophysics, School of Medicine, University of California, Los Angeles, California

THIS paper reports the presence in pituitary extracts of a material which inhibits the milk clotting activity of crystalline pepsin. The inhibitor was found concentrated in commercial preparations of thyrotropin, with increased inhibitor activity present in preparations most potent with respect to thyrotropin activity. Recently, at this institution, a chromatographic method for the partial purification of thyrotropin was developed, and a further fractionation by counter-current distribution has also been accomplished (1, 2). Assays of samples made available to us from these fractionation procedures[2] provide strong evidence that the pepsin inhibitor is a separate entity from material causing stimulation of P^{32}-uptake in chick thyroids. Details of the method used for assaying pepsin inhibition are presented.

MATERIALS AND METHODS

The method used for measuring pepsin inhibition is an adaptation of a uropepsin assay (3). Determinations are carried out at 37° C in a water bath in the presence of 1.5N sodium acetate buffer at pH 4.9. The substrate is fresh homogenized milk. A stock enzyme solution is prepared by dissolving 5 mg. crystalline pepsin[3] in 500 ml. 0.1N HCl. Just before use, an aliquot of the stock enzyme solution is standardized by pipetting 0.9 ml. distilled water, 1 ml. buffer and 0.5 ml. *buffered* milk (equal vols. milk and buffer) into a 120×12 ml. test tube. After warming to 37°, 0.1 ml. of cold enzyme is added rapidly, a stop watch is started, the contents of the tube mixed quickly and returned to the water bath. The tube is shaken gently every 20 seconds. The end point is recognized as a fine precipitate appearing just above the surface of the mixture. The stock enzyme solution is appropriately diluted to give a sharp end point in exactly 90 seconds. Once diluted, the enzyme will retain potency for about 2 hours at 0°.

To determine enzyme inhibition, aqueous solutions of unknown are substituted for a portion or all of the water, and the increased time necessary for the appearance of the end point is noted. During this procedure, tubes are observed every 10 seconds, but shaken only at 30-second intervals, since the end point becomes less discernible as inhibition time increases. For accuracy, concentrations of the unknown should be adjusted to produce an end point falling between 100 and 400 seconds. One hundred seconds of inhibition (190 seconds after the enzyme has been added) are designated arbitrarily as one "inhibitor unit."

Received January 29, 1957.

[1] Present address: Institute of Technology, Zurich University, Zurich, Switzerland.

[2] J. G. Pierce, M. E. Carsten and J. F. Nyc of the Department of Physiological Chemistry very kindly supplied the samples and carried out the P^{32}-uptake assays.

[3] Armour and Worthington crystalline pepsin gave comparable results.

The pH range in which inhibition can be tested is from 4.9 to 5.7. The former pH has been selected for this assay procedure because of the relative stability of both enzyme and substrate.

RESULTS

Small and variable amounts of inhibitor were present in aqueous extracts of several commerical pituitary gland preparations.[4] In the commerical thyrotropin preparations tested (Table 1) pepsin inhibiting activity was high and paralleled their thyrotropic potency. United States Pharmacopeia Thyrotropin Reference Standard[5] and different lots of Armour's Thytropar gave consistent and comparable values, and a linear relationship was obtained when inhibition was plotted against units of thyrotropin activity.

TABLE 1. PEPSIN INHIBITOR CONTENT OF VARIOUS PREPARATIONS FROM PITUITARY

Material assayed	Trophic activity units/mg.[1]	Mg. used in inhibitor assay	Pepsin inhibition units/mg.[2]
Thyrotropin			
U.S.P. Thyrotropin Ref. St.[3]	0.074 U.S.P.	1.36	0.8
Armour Thytropar, lot 2211	1.2 U.S.P.	.12	13
Armour Thytropar, lot 2308	1.3 U.S.P.	.13	15
Parke-Davis Thyrotropin, lot 50P4	2–2.5 U.S.P.	.10	28
Growth Hormone			
Armour Somar, lot 308	n[4]	2.5	<1
Armour G. H., lot R377237	n	1.0	<1
Armour G. H., lot 264-159A	n	.8	<1
Armour G. H., lot 300-160	n	2.5	<1
Corticotropin			
Armour corticotropin, lot L60612	3.4 I.U.[5]	2.0	<1
Armour Acthar, lot J25810	4.7 I.U.	1.0	<1
United, Actrope, lot 114111	n	1.0	1.7
Luteinizing Hormone			
Armour L. H., lot R377242H	7 mg. Armour St.	1.0	<1
Armour L. H., lot 22780	n	.2	12
Follicle Stimulating Hormone			
Armour F.S.H., lot R377245	1 Armour U.	1.0	2.6
Squibb Gonadotropin 4E77722	21 Rat U.	2	<1
Searle Gonadophysin, lot 3467	20 Rat U.	.5	3.4
Upjohn Gonadogen EC361BD (PMS)[6]	24 I.U.	90	0
Ayerst Equinex #490 (PMS)	61 I.U.	13	0
Parke-Davis Antuitrin S[7]	1350 U.	.1	0
Melanophore Stimulating Hormone			
Armour, lot 600-295-12	n	2.5	<1
Armour, lot D216-155C	n	1.5	1.13

[1] As stated by manufacturer for particular activity indicated by the name of the preparation.
[2] 1 Inhibitor Unit = 100 secs. inhibition.
[3] U. S. Pharmacopeia.
[4] n = not known.
[5] International Units.
[6] PMS = pregnant mare's serum.
[7] Antuitrin S = chorionic gonadotropin.

[4] Armour Ant. Pit. list 19925 and Post. Pit. list 20522. Mallenhauer whole Pit. Lot 399.47 and Parke, Davis Pit. Body, whole gland, M851 B.

[5] Obtained from United States Pharmacopeia Reference Standards, 46 Park Avenue, New York 16, New York. This material was stated to contain approximately 1.48 U.S.P. units per tablet or 0.074 unit per mg. of tablet.

The high pepsin inhibition obtained for thyrotropin preparations was in contrast to many other commerical pituitary hormone preparations assayed (Table 1). Although some of the gonadotropins showed appreciable amounts of inhibitor, these amounts were not related to gonadotropic potency. Furthermore, extra-pituitary gonadotropins of placental origin failed to show pepsin inhibition.

In addition to the commerical preparations assayed, pepsin inhibitor assays were made on thyrotropin concentrates prepared at this institution by Pierce and Nyc (1). These authors used cation-exchange chromatography to obtain concentrates which contained approximately 5U.S.P. units/mg. when assayed against the U.S.P. Reference Standard.[5] As an assay method they used P^{32}-uptake in chicks (4). The starting material for their chromatographic experiments was prepared by the initial extraction and acetone precipitations of Ciereszko (5). Variable pepsin inhibiting activity was found in these starting preparations with values ranging from 2–10 inhibitor units/mg. Assays of various fractions obtained by chromatography showed the inhibiting activity to have similar, but not completely parallel, chromatographic properties to those of the material stimulating P^{32}-uptake. Increased amounts of pepsin inhibiting activity were found in the final products with the units of pepsin inhibition depending on the units present in the particular starting preparation used. This occurrence of variable ratios of inhibiting activity to P^{32}-uptake activity suggested the presence of two active entities. Recent counter-current distribution experiments carried out by Pierce and Carsten present strong evidence for this conclusion (2). By distribution between *n*-butanol and *p*-toluene sulfonic acid, two main fractions were obtained from the material prepared by ion-exchange; one fraction strongly favoring the aqueous, the other the organic phase. The latter fraction, when assayed by us, was high in pepsin-inhibiting activity (one sample containing 60–70 inhibitor units/mg.), but was essentially free of P^{32}-uptake activity. The material favoring the aqueous phase, active in the P^{32}-uptake assay, was free of pepsin inhibiting activity.

In order to determine the specificity of the inhibitor, commerical thyrotropin preparations were also tested against uropepsin (prepared by activation of urinary pepsinogen (3)) and two other milk-coagulating enzymes. Using the technique described above, assays were made with uropepsin and calf rennin at pH 4.9, and with both rennin and chymotrypsin at pH 6.65. The technique employed at the latter pH was presented earlier (6). The results, summarized in Table 2, show that of the enzymes tested, only pepsin and uropepsin were inhibited significantly.

DISCUSSION

There have been no previous reports of a specific enzyme inhibitor in pituitary tissue, although enzymes with proteolytic activity have been

TABLE 2. SPECIFICITY OF ENZYME INHIBITOR

Source of inhibitor	Units of TSH activity per mg.[1]	Mg. used in assay	Enzyme	pH	Inhibition in seconds
U.S.P. Thyrotropin Reference Standard	0.074	1.36	Pepsin[2]	4.9	100
	0.074	1.36	Uropepsin[3]	4.9	105
	0.074	5.32	Rennin	4.9	0
	0.074	6.67	Rennin	6.65	0
	0.074	6.67	Chymotrypsin	6.65	0
Parke-Davis Thyrotropin, lot 50P4	2–2.5	.1	Pepsin	4.9	280
	2–2.5	.1	Rennin	4.9	10
	2–2.5	.9	Rennin	4.9	35
	2–2.5	.5	Rennin	6.65	0
	2–2.5	.5	Chymotrypsin	6.65	0
Armour Thytropar, lot 2211	1.2	.2	Pepsin	4.9	280
	1.2	.2	Uropepsin	4.9	300
	1.2	.5	Rennin	4.9	15

[1] TSH activity as stated by manufacturer.
[2] Armour's porcine pepsin was used.
[3] Human uropepsin, made by activation of urine pepsinogen (3).

obtained from whole gland and partially purified pituitary extracts (7) Sulfonated mucopolysaccharides inhibit pepsin (8) but when assayed by the method presented in this paper, an equal weight of heparin had only approximately $\frac{1}{5}$ the activity of Armour's Thytropar. Herriott has described the isolation and crystallization of a polypeptide obtained by the acid hydrolysis of pepsinogen and which has powerful inhibiting action on both the proteolytic and milk-coagulating properties of pepsin (9). Any possible relationship between the chemical nature of this polypeptide and the inhibitor present in pituitary extracts remains to be investigated.

Dialyzed sera from human, rabbit and rat blood showed considerable pepsin inhibition. In an experiment carried out prior to the separation of the two activities by counter-current distribution, an attempt was made to correlate pepsin inhibiting activity of serum with levels of circulating thyrotropin. This was unsuccessful, however, since an intracardiac injection of 10 units of Armour's Thytropar to a rat did not produce an increase in the inhibiting value of the serum when measured at 1 and 72 hours following administration.

SUMMARY

A material has been found in pituitary extracts which inhibits the milk coagulative property of pepsin and uropepsin but fails to act on rennin and chymotrypsin. In several commercial thyrotropin preparations, the inhibiting potency was found to parallel the thyrotropic potency. Inhibiting activity was also present in thyrotropin concentrates prepared by chromatography. However, assays of preparations further fractionated by counter-current distribution show that the inhibiting activity is clearly separable from material stimulating the uptake of P^{32}.

Acknowledgments

The authors wish to express appreciation to Doctors John G. Pierce, Mary E. Carsten and Joseph F. Nyc of the Department of Physiological Chemistry for their generous cooperation and assistance, as well as to thank Doctor Warner Florsheim, Radioisotope Service, Long Beach Veterans Administration Hospital and Doctor Irby Bunding of Armour for supplying some of the hormone preparations used.

REFERENCES

1. Pierce, J. G. and J. F. Nyc: *J. Biol. Chem.* **222:** 777. 1956.
2. Carsten, M. E. and J. G. Pierce: *Fed. Proc.*, in press, 1957.
3. West, P. M., F. W. Ellis and B. L. Scott: *J. Lab. and Clin. Med.* **39:** 159. 1952.
4. Greenspan, F. S., J. P. Kriss, L. E. Moses and W. Lew: *Endocrinology* **58:** 767. 1956.
5. Ciereszko, L. S.: *J. Biol. Chem.* **160:** 585. 1954.
6. West, P. M. and J. Hilliard: *Proc. Soc. Exp. Biol. and Med.* **71:** 169. 1949.
7. Adams, E.: *Fed. Proc.* **10:** 153. 1951.
8. Marini, M. and S. Levey: *Proc. Soc. Exp. Biol. and Med.* **88:** 611. 1955.
9. Herriott, R. M.: *J. Gen. Physiol.* **24:** 325. 1941.

NOTES AND COMMENTS

GONADOTROPHIN CONTENT OF PITUITARY OF PREGNANT AND PSEUDOPREGNANT RATS FOLLOWING SINGLE INJECTION OF ERGOTOXINE[1]

The termination, in the rat, of early pregnancy and psuedopregnancy (1) and deciduoma development (2) by single subcutaneous injections of ergotoxine is associated with the appearance of estrous vaginal smears within 96 hours after the injection. Administration of supplemental progesterone reverses these ergotoxine effects (2,3). It thus appears that the ergot complex either increases systemic estrogen or depresses systemic progesterone (4). However, in assays carried out on spayed adult rats and on immature intact female rats we found no evidence of estrogenic or gonadotrophic activity in ergotoxine (5). Fitzhugh *et al.* (6) showed that long-term ergot feeding produced "luteinized" ovaries in the rat, but the ovary of the ergotoxine-treated rat responded to endogenous pituitary stimulation (7). The possibility exists that the ergot drug acts on or via the pituitary gland.

In the course of investigations of the site and mechanism of the ergotoxine action on the pregnant and pseudopregnant rat, we assayed the pituitary glands of ergotoxine-treated rats for alterations of gonadotrophin content.

METHODS AND RESULTS

Colony-bred, originally Wistar albino rats (180–200 gm.) with regular, established estrous cycles were either mated with fertile males, or made pseudopregnant by electrical stimulation of the cervix. Thirty pseudopregnant rats served as donors for assay of gonadotrophins (FSH & LH); 15 of the donors received 1.5 mg. ergotoxine (in 0.2 ml. ethanol 95%) (s.c.) on the 4th day of pseudopregnancy (Day 4 of leucocyte smear); 15 control donors received alcohol injections. Twenty hours after the injection the donors were sacrificed, pituitaries of ergotoxine-treated donors pooled and macerated with cold saline to make 5 ml, divided into 5 aliquot portions and cooled to −30° C. Equivalents of 3 whole glands were injected into each of 5 immature female rats (24-day old) over a period of 5 days. Pituitaries of control donors were treated and administered in the same way. Immature test animals, recipients of ergotoxine-pituitaries, control alcohol-pituitaries and non-treated, were autopsied one day after the final injection (age, 29 days); ovaries and uteri were carefully trimmed and weighed. No significant differences were found between ovarian or uterine weights of the immature recipients, of pituitaries of ergotoxine-treated pseudopregnant donors, and recipients of control (alcohol) pseudopregnant pituitaries (Table 1).

Ten female rats were injected with 1.5 mg ergotoxine on the 20th day of pregnancy and 24 hours later, their pituitaries were removed and assayed for prolactin (8). Ten alcohol-treated pregnant rats served as donors for control assays. The assay was conducted by Dr. Joseph Meites on pigeon crop glands. There was no difference between prolactin content of pituitaries of parturient rats pretreated with ergotoxine and of control pituitaries.

DISCUSSION

Dosages of ergotoxine which terminate early pregnancy and pseudopregnancy, and suppress deciduoma development, failed to modify the FSH, or LH content of the pituitaries of the pseudopregnant or pregnant rat. Actual modifications which could have been detected would be: increased secretion greater than that released, increased storage, or depletion. The possibility that pituitary was producing more gonadotrophin but at the same time releasing it, thus keeping the pituitary content unchanged, does exist, and these experiments would not detect such a condition. The pituitary glands were removed from the ergotoxine-treated rats 24 hours after injection, which is about a day before the

Received January 24, 1957.

[1] Supported, in part, by grant-in-aid from Population Council, New York, and Sandoz, Basle.

TABLE 1. OVARIAN AND UTERINE WEIGHTS OF IMMATURE RATS FOLLOWING INJECTION OF PITUITARY OF ERGOTOXINE-TREATED PSEUDO-PREGNANT RAT

Pituitary donors		Recipient immature rats		
Type	No.	No.	Ovary (mg. ±S.e.m.)	Uterus (mg. ±S.e.m.)
Ergotoxine-treated pseudopregnant	15	5	18.9±1.24	60.9±5.04
Alcohol-treated pseudopregnant	15	5	17.4±1.71	54.8±3.91
			$t=0.626$ $0.5<P<0.6$	$t=0.882$ $P=0.4$
None	—	5	14.2±1.81	31.5±3.5

ergotoxine effect of pregnancy and pseudopregnancy termination, and thus early enough to examine the hypophysis during time of expected changes which act on target organs later. The timing for pituitary assay after ergotoxine injection would appear to be a critical factor. Examination of gonadotrophin content at the time when the ergotoxine effect is already manifested may be either too late, or give false values which reflect pituitary response to the ergotoxine-altered uterine status. The gonadotrophins were assayed on the genital tract of female rats, and on crop glands of pigeons.

SUMMARY

The gonadotrophins of pituitaries of ergotoxine-treated pseudopregnant and pregnant rats were assayed on immature female rats, and on crop glands of pigeons. There were no differences between the FSH or LH of pituitaries of ergotoxine-treated and control donors.

Acknowledgment

It is a pleasure to acknowledge my appreciation to Mr. Joseph Shalom for his constant assistance.

M. C. SHELESNYAK

Department of Experimental Biology
Weizmann Institute of Science
Rehovoth, Israel

REFERENCES

1. SHELESNYAK, M. C.: *Amer. J. Physiol.* **180**: 47. 1955.
2. SHELESNYAK, M. C.: *Amer. J. Physiol.* **179**: 301. 1954.
3. SHELESNYAK, M. C.: *Acta Endocrinol.* **23**: 151. 1956.
4. SHELESNYAK, M. C.: *Rec. Prog. Hormone Res.* **13**: in press 1957.
5. SHELESNYAK, M. C.: *Proc. Soc. Exper. Biol. & Med.* **94**: in press 1957.
6. FITZHUGH, O. G., A. A. NELSON AND H. O. CALVERY: *J. Exp. Pharm. & Therap.* **82**: 364. 1944.
7. SHELESNYAK, M. C.: (unpublished).
8. BERGMAN, A. J., J. MEITES AND C. W. TURNER: *Endocrinology* **26**: 716. 1940.

ON THE PROGESTATIONAL ACTIVITY OF 17α-ETHYNYL-17-HYDROXY-5(10)-ESTREN-3-ONE (NORETHYNODREL)

Hertz *et al.* (1) reported that 17α-ethynyl-19-nortestosterone is an orally effective progestational agent in the rabbit, being about 5 times as active as the normal homologue. Since 17α-ethynyl-17-hydroxy-5(10)-estren-3-one (norethynodrel) (SC-4642) differs structurally from 17α-ethynyl-19-nortestosterone only in the position of the double bond in the A ring, it might also be expected to produce a progestational response. In a recent paper, Pincus *et al.* (2) reported it to be half as active as progesterone when administered subcutaneously in the Clauberg assay. On oral administration they found this compound to be inactive in the Clauberg test although they showed activity in ovulation inhibiting tests in rabbits and in delaying fertile matings in rats after oral treatment.

In this laboratory norethynodrel has been examined for its ability to produce endometrial gland development in the estrogen-primed spayed or immature rabbit with 3 routes of administration: subcutaneous, oral, and intrauterine. The results seem sufficiently unusual to merit reporting at this time.

Rabbits spayed 2 weeks previously, were primed with 6 daily subcutaneous injections of 5 μg. of estrone each. On the day after the last injection, the uterus was exposed. A 1 inch segment of one horn was tied off and injected with the test compound in 0.1 ml. corn oil after the method of McGinty *et al.* (3). A similar segment of the contralateral horn was treated with oil and served as a control. The animals were sacrificed 72 hrs. later and the uterine segments were prepared for histological study. They were graded from +1 to +4 on the basis of endometrial gland development. The average rating for 545 estrogen-primed control uteri was 1.04. In general, doses which produce an average response of 2 or over are considered active. Comparative results obtained with progesterone and norethynodrel are shown in Table A1 from which it is evident that the latter is less than 1/200 as active as progesterone in this assay.

Immature rabbits, weighing about 1 kg. each, were primed with 6 daily subcutaneous injections of 5 μg. of estradiol. Starting on the day after the last priming dose, they were treated daily for 5 days with the test compound either subcutaneously or orally. The animals were sacrificed on the day after the last injection. The results are shown in Tables 1B and 1C respectively. The variability was marked and maximal responses were not seen with norethynodrol. Administered subcutaneously, the compound was somewhat less potent than progesterone. However, administered orally it appears to be 10 to 25 times as active as oral progesterone. Furthermore the average response to norethynodrel after oral administration was greater than the average response to the same dose of this compound injected subcutaneously in oil.

In the intrauterine assay, the addition of 1 μg. or more of estrone to 5 μg. of progesterone will completely block the endometrial gland response to progesterone. Smaller doses of estrone do not show this inhibitory effect (4). Norethynodrel has definite estrogenic effects. At minimal effective doses it was found to be about 7% as active as estrone in inducing uterine growth in the immature mouse and 3–5% as effective as estrone in promoting vaginal cornification in the adult spayed rat. When injected into the lumen of the uterus, this estrogenic activity may prevent the manifestation of the inherent progesterone-like action of the compound.

SUMMARY

On intrauterine administration, 17α-ethynyl-17-hydroxy-5(10)-estren-3-one (norethynodrel) proved inactive as a progestational agent in the rabbit. When given sub-

Received February 9, 1957.

TABLE 1. AVERAGE ENDOMETRIAL RESPONSES TO 17α-ETHYNYL-17-HYDROXY-5(10)-ESTREN-3-ONE (NORETHYNODROL) AND PROGESTERONE IN THE RABBIT

Compound	Dose*						
A. Intrauterine Assay, McGinty							
	100	10	5	2	1	0.5	0.2
Progesterone		3.8 (6)†	3.0 (15)	2.8 (16)	2.6 (13)	2.3 (7)	1.5 (4)
Norethynodrel	1.1 (7)	1.1 (7)	1.7 (3)	1 (1)	1.3 (7)	1 (1)	
B. Subcutaneous, Clauberg							
	5	1	0.5	0.2	0.1	0.05	0.02
Progesterone		3.9 (4)	3.8 (2)	3.4 (4)	3.1 (7)	2.9 (8)	1.1 (8)
Norethynodrel	2.0 (2)	1.2 (4)	1.9 (6)	1.9 (20)	1.6 (7)	1.0 (6)	1.0 (4)
C. Oral, Clauberg							
	20	10	5	2	1	0.5	0.2
Progesterone	3.3 (3)	2.5 (4)	2.3 (4)	1.3 (4)			
Norethynodrel		2.5 (2)	2.2 (3)	2.7 (3)	3.1 (4)	2.8 (4)	2.4 (8)

* In the intrauterine assay, this represents a single dose in μg.; in the Clauberg, the dose in mg. administered daily for 5 days.

† The number in parentheses is the number of rabbits at that dose.

cutaneously, some response was obtained but oral administration appeared to give greater responses. Orally it was 10 to 25 times as effective as progesterone administered by the same route.

FRANCIS J. SAUNDERS, RICHARD A. EDGREN AND VICTOR A. DRILL
Division of Biological Research
G. D. Searle & Co.
Chicago, Illinois

REFERENCES

1. HERTZ, R., W. TULLNER AND E. RAFFELT. *Endocrinology* **54**: 228. 1954.
2. PINCUS, G., M. C. CHANG, M. X. ZARROW, E. S. E. HAFEZ AND A. MERRILL. *Endocrinology* **59**: 695. 1956.
3. MCGINTY, D. A., C. P. ANDERSON AND N. B. MCCULLOGH. *Endocrinology* **24**: 289. 1939.
4. SAUNDERS, F. J. Unpublished data.

BOOK REVIEW

MEMOIRS OF THE SOCIETY FOR ENDOCRINOLOGY. No. 4. The Comparative Endocrinology of Vertebrates. Part 1. The Comparative Physiology of Reproduction and the Effects of Sex Hormones in Vertebrates. I. Chester Jones and P. Eckstein, editors. Cambridge University Press, 1955. 253 pp., illustrations.

This book is the first of two volumes of a symposium on the comparative endocrinology of vertebrates, held at the University of Liverpool in July, 1954. The initiator of this symposium was Dr. I. Chester Jones, Professor of Zoology at the University of Liverpool. There are 14 chapters by various Continental and American authorities, and discussions of these chapters by most of the 58 participants. Opening and closing remarks were appropriately and enthusiastically made by Dr. S. Zuckerman, chairman of the symposium.

The book seems to be divided into three parts:

The plan of the first part is orderly, for generally a chapter is devoted to reproductive functions in either or both sexes of the various vertebrate classes from teleosts through mammals. However, I wondered if a better arrangement wouldn't have been possible by handling the male and the female sexes separately and treating each as a continuum throughout the vertebrate series. Also, the omission of a chapter on reproduction in the elasmobranchs puzzled me; sufficient knowledge is available for an instructive contribution.

The mid-part of the book consists of two chapters, one on the evolution of viviparity and another on gonadotropins of vertebrates. Both chapters seem misplaced, for had the arrangement suggested been followed, the chapter on gonadotropins might well have come as the introductory chapter for the first portion, and the review of viviparity could have logically followed the comparative survey of the female reproductive tract.

The last part contains five chapters which, in contrast to the first part, seem haphazardly thrown together. This part was supposed to have dealt with the effects of sex hormones in various classes of vertebrates. Only two of the chapters qualify for this theme. A third chapter is obviously a mixture, and the two remaining chapters deal with fetal endocrinology. These two chapters, by Jost and Gallien respectively, seem to me to have no place in this symposium. They are, however, of sufficient stature to constitute a nucleus for another symposium on comparative fetal endocrinology which, with contributions by Wells, Forbes, Burns, Wolff and Witschi, would be timely.

The contents of this volume are of great interest. Some of the data are fascinating and some of the reported experimental work was remarkable. The relentless progress of scientific information in the field of lower vertebrate reproduction in the last 25 years is evident and it has resulted in the formulation of new concepts in endocrinology. Despite this generally favorable impression, I found the chapters, individually, to be uneven in style, context, coverage, readability and lucidity.

The opening paper of the first part of the book is Hoar's chapter on reproduction in teleosts, an excellent general survey of the extreme variability of reproduction in fishes. Although this account gives much information about the reactions of the teleost reproductive tract to known sex steroids, practically nothing is known of the hormones that the gonads of fishes presumably manufacture. It is also evident that the work done on the hormonal control of the reproductive system in general has been heavily influenced by preconceived notions borrowed from mammalian endocrinology. For example, it was logical to believe that spawning behavior in fishes is induced by testicular steroids, considering the work on sex behavior of mammals, but this belief receives a jolt from Pick-

ford's recent demonstration that a pituitary principle (probably from the posterior lobe, at that) directly induces spawning reflexes in Fundulus. The idea of a direct effect of pituitary principles on somatic structures, without the mediation of the gonads, crops up in several chapters in this book.

Unlike the first chapter, which is a comprehensive review, the contribution by the van Oordts is mainly concerned with experiments carried out in their laboratory. These experiments dealt with the seasonal sensitivity of the anuran testis to exogenous gonadotropin. One conclusion—that spermatogenesis can start only under the influence of gonadotropic hormones—reminded me of some experiments I did on Rana pipiens in 1935. The frogs were hypophysectomized in midsummer and were maintained for 6 months. Injection of testosterone into these 6-month hypophysectomized frogs caused intense spermatogenesis.

Smith's chapter on reproduction in female Amphibia is a good summary of the regulatory mechanisms controlling the amphibian ovary. Now, for the first time, the important concept appears that an end organ—for example, the oviducal gland—is under the dual control of the pituitary and the ovary, a concept that may have to be considered in mammalian endocrinology if it is true that prolactin acts directly on the prostate of the rat. Another point which is now established is that the reproductive system is not the only system that is activated in seasonal breeders. Other portions (pituitary-thyroid) of the endocrine system may also be excited and thus affect gonadal function. It is disappointing that new information on the nature of preovulatory and postovulatory corpora lutea is absent.

The subject of reproduction in the reptilia is handled by Kehl and Combescot in taxonomic departments (Sauria, Ophidia, Chelonia and Crocodilia). This chapter is terse and fully documented but it lacks interest and generalization. The work in reptiles as a whole does not have the mark of originality, since much of the experimental material to date is a repetition of mammalian experiments. This lack is unfortunate since some aspects of reptilian reproduction (placentation, viviparity) seem uniquely suitable for original study. Also, it is curious that Kehl and Combescot did not participate in the discussion of their own paper. Certainly a number of the participants could have acted as interpreters; perhaps a translation of their paper was read in their absence—the editors do not explain.

Marshall has written a critical and superbly colorful account (as only the English can write English) of reproduction in male birds—by far the most interestingly written chapter. The importance of photostimulation in sexual periodicity having been cut down to what appears to be its proper dimensions, Marshall discusses activation of the adenohypophysis and pituitary-gonadal relationships. He also reports certain of his recent research interests. These consisted of shooting wild birds engaged in post-nuptial sexual behavior in the hope of determining the source of the hormones responsible for this behavior. But in order to be sure of what constitutes indubitable sexual behavior, Marshall listed a number of birds, including the male Australian magpie, which "commit the avian equivalent of rape, an activity which should satisfy the most stringent criteria." So, he induced Robinson of Australia to agree to shoot such rapacious birds in the act and to send on their gonads. Unfortunately, the only thing that Robinson was able to send on was the complaint that whenever he appeared on the scene with a shotgun, the rapacious (but sagacious) birds did not perform. Two other phenomena in this account are absorbing—the steatogenesis of the tubule and the appearance of a new crop of Leydig cells in hypophysectomized animals. The latter phenomenon is in marked contrast to the seemingly utter dependence of Leydig cells of primates on the pituitary for their differentiation and morphologic integrity. The discussion of this paper might have been a gem; but the chairman turned over the meeting to Benoit who lectured on

his own considerable and important contributions to the field, many of which already had been covered in Marshall's paper.

Breneman's contribution centered on the relation between the pituitaries and the ovaries of birds, on pituitary cytology, on types of gonadotropins in the bird pituitary, on the response of the ovary and on the nature of ovarian hormones. The possible steroid hormones produced by the avian ovary are not known although it is claimed that androgenic, estrogenic and luteoid steroids are secreted. There is a discordant note, which blossoms out later, that the avian pituitary contains FSH, LH and ICSH.

The last chapter in this first part is by Eckstein and Zuckerman—a thought-provoking essay on the sex cycles in mammals. The authors point out that the accepted concept of dividing mammalian cycles sharply into opposing types may be wrong, and that these diametrically opposing types may be really the extremes of a continuous variation. Thus, sex cycles of mammals may differ in degree but not in kind. This is perhaps the best organized account in the book.

As has been said, the mid-portion of the book contains two very different chapters. The first of these is an excellent account of the evolution of viviparity by Mathew, supplemented by Amoroso's authoritative discussion. The second is a provocative exposition of vertebrate gonadotropins by Witschi, who expressed the belief that LH and ICSH are different hormones. The identity of LH and ICSH was the cause of a transcontinental biochemical war between Fevold and Li, generals of the Eastern and Western forces, respectively. Li's surrender put an end to the concept that ICSH and LH are separate hormones, but now Witschi seems to have breathed some life into the old corpse. Whether or not anything resembling a resurrection will occur remains to be seen. Witschi also expressed the belief that it is LH that directly acts on the cock plumage of the weaver finch, but such action would seem to depend on the chemical cleanliness of preparations of LH. In the same way, the "contraction" of xanthophores in Amphibia after injection of Armour's FSH does not mean that the response is necessarily and actually due to FSH. In any case, Witschi's concept—that a gonadotropin may act directly on a nongonadal structure—is highly important.

The last part of the volume begins with Dodd's survey of reproduction in urochordates, cyclostomes, elasmobranchs and teleosts. Parts of this survey would fit well with the material in the first part of the book. Dodd's clear thinking and restraining influence are evident in his chapter as well as in the discussions. He points out that large gaps exist in knowledge of reproductive processes in fishes, for only a handful of species out of 20,000 have been examined in sufficient detail. To the suspected homology of the ascidian neural complex and the vertebrate pituitary, Dodd correctly gives a verdict of not proved. Fraps discusses the evidence for the concept that a progestin trips the ovulatory mechanism in birds, and the evidence favors the possibility that progesterone is actually the hormone secreted. Leathem's account is a review of various actions of sex hormones in mammals.

By way of general comment, I probably should say that I was irritated by two features of the book. The first was the poor quality of some of the graphs and illustrations, for which the authors and not the publishers are to blame. The avalanche of scientific publications today prevents anyone's keeping abreast of his own field, so that even interested readers will not stop to puzzle over a graph that is not clear at first glance, nor will they try to fathom the meaning of co-ordinates if, indeed, they can be found. Some of the graphs have entirely too many curves, and some material given in tables would have been much more informative if expressed as graphs. Such defects reflect lack of use of the editorial whip, and no amount of disclaiming on the part of the editors (as is the English custom—cf. footnote, page 5) can relieve them of responsibility.

The second matter that was irritating to me was the manner in which the discussions

were edited, or rather, not edited. In one place, the discussion winds up in a *non sequitur*, and in another area, even after rereading, I came to the conclusion that part of the Galley proof must have been lost in the mail. In still another discussion, the remarks concerning the elegance of biochemical vis-à-vis morphologic work may have been enjoyed by those present as incomparable repartee—but as the discussion appears in print, it falls flat. Concerning discussions, anyone who has had the dubious distinction of editing them is always faced with the problem of how to get the spirit of the discussions into print. Obviously, an editor cannot print the discussions verbatim as they are recorded by machine, for then none of the discussants would be foolish enough ever to take part in another symposium. On the other hand, a discussion cannot be edited to the point where it loses spontaneity. Somewhere between, with a comfortable standard deviation, lies the golden mean and I would guess that the editors of the *Laurentian Hormone Conference Proceedings* come as close to this mean as any. If they set the standard, the present editors have not measured up to it.

Apart from these somewhat testy comments (which I trust will not forever bar me from the Commonwealth), a series of remarks made in the course of the symposium seem to me to qualify only for the scientific equivalent of the "How's That Again? "department of the *New Yorker* magazine. The hormone under discussion, despite the mangling it underwent, is what has recently been termed in clinical and chemical literature the "melanocyte stimulating hormone" and is known more intimately by its abbreviation, MSH. This term is not correct and, therefore, is not acceptable to zoologists who know about such things as the pigmentary effector system. The following four quotations are examples of how editorial scrutiny could have prevented the bungling:

1. Page 165, "FSH (Armour) causes contraction of yellow chromatophores (guanophores); MEF is the melanophore expanding factor. Different reactions and different hormones are involved. MEF is a contaminant of FSH (Armour)."

2. Page 185, "The xanthophore expanding principle which I found in this gland [neural] may perhaps be a FSH."

3. Page 186, "With regard to your suggestion that the xanthophore-expanding principle which you found in the ascidian neural gland might perhaps be a FSH, I was under the impression that . . . a xanthophore contracting property associated with the various gonadotropins. . . ."

4. Page 250, ". . . apparently pure mammalian FSH may have a very potent melanophore-expanding effect in Amphibia. FSH is thus MEH; and, no doubt, the contrary proposition also applies."

I trust these lines are not plucked too badly out of context. In any case, it is clear that yellow and white chromatophores have been confused, that concentration and dispersion of chromatophore pigment have been confused, and it is also clear that the last remark is not altogether clear.

This book left me with the general impression that there is a large amount of work to be done on the comparative physiology of reproduction. That so much remains to be done is due partly to the overwhelming influence of mammalian, and more particularly human, endocrinology on investigative activity on lower vertebrates. Shortly after the turn of the century, P. E. Smith and B. Allen performed exemplary experiments on the amphibian tadpole. It is interesting to conjecture what the state of endocrinology of the lower vertebrates might have been had Smith and Allen's basic discoveries been followed by a full-scale attack on the lower forms. Such an attack never was launched, probably because the emphasis shifted completely to mammals. Hence, the development of mammalian, including human, endocrinology was greatly accelerated, but might it not have been even further advanced? Hormones and tissues must have been subjected to evolutionary experiment. Mammals did not invent the hormones, and knotty prob-

lems both of man and lower mammals might not seem so knotty had a broad base of scientific knowledge been built up regarding the lower vertebrates. This book attests to the fact that precious little independent and original work has been done with lower forms. Perhaps it is yet not too late, for now powerful tools of chemistry and physics can be applied to problems of lower vertebrate endocrinology which only a few years ago seemed impossible to solve. In particular, such problems as the nature and kinds of hormones produced by the gonads and other glands of lower vertebrates can be solved by chromatographic, isotopic, spectrophotometric methods, and by use of such resolving tools as countercurrent partition and electrophoresis. Minute amounts of material are no longer an obstacle to identification. Although considerable progress has been made, there are yet not enough facts, mechanisms, integrations, interrelations and concepts in comparative endocrinology to constitute an established branch of scientific inquiry. The advances made to date are gratifying, and are evident in this book, but as for a "comparative endocrinology," this book is not it.

A. Albert, M.D.
Mayo Clinic and Mayo Foundation
Rochester, Minnesota

THE ENDOCRINE SOCIETY

1957 AWARDS

The selection of the recipients of the awards of The Endocrine Society is made by a Committee appointed by the Council of the Society. These awards and fellowships carry no obligation by the recipient to the Society or to the donors.

Medal of The Endocrine Society

In 1954 the Council of the Society voted to establish a medal and an honorarium of $1,000 to be given to an individual for work of special distinction in endocrinology. The recipient shall be chosen from nominations presented by members of the Society and is limited to citizens of the United States and Canada. Dr. Carl R. Moore was the recipient in 1955 and Dr. Frederick L. Hisaw in 1956.

The Ciba Award

The Ciba Award, to recognize the meritorious accomplishments of an investigator not more than 35 years of age in the field of clinical or preclinical endocrinology, was established in 1942, but no recipient was selected in 1942 or 1943. In 1944 the Award was presented to Dr. E. B. Astwood; 1945—Dr. Jane A. Russell; 1946—Dr. Martin M. Hoffman; 1947—Dr. Choh Hao Li; 1948—Dr. Carl Heller; 1949—Dr. George Sayers; 1950—Dr. Oscar M. Hechter; 1951—Dr. Albert Segaloff; 1952—Dr. Seymour Lieberman; 1953—Dr. Sidney Roberts and Dr. Clara M. Szego (Mrs. Roberts); 1954—Dr. I. M. Rosenberg; 1955—Dr. Jack Gross; 1956—Dr. Alfred M. Bongiovanni. Prior to 1952 the Award was $1,200. It is now $1,800. If within twenty-four months of the date of the award, the recipient should choose to use it toward further study in a laboratory other than that in which he is at present working, it will be increased to $2,500.

The Ayerst and the Squibb Fellowships

The Ayerst Fellowship was established in 1947 and the Squibb Fellowship in 1956. They are designed to assist men or women of exceptional promise in furthering their advancement towards a career in endocrinology. Each Fellowship is awarded on alternate years and the stipend, which will not exceed $5,000, may be divided into two Fellowships in varying amounts in accordance with the qualifications of the appointees. Individuals possessing the M.D. or Ph.D. degree, or candidates for either of these degrees, are eligible for appointment.

Applicants must submit the following information:

1. Evidence of scientific ability as attested by studies completed or in progress.
2. Recommendations from individuals familiar with the candidate and his work.
3. A proposed program of study.
4. Acceptance of the individual by the head of the department in which the Fellowship will be held.
5. A statement that he or she will serve full time if awarded a Fellowship. A small amount of time (10 to 15 per cent) may be spent in course work or participation in teaching, the latter purely on a voluntary basis.

The Schering and The Upjohn Scholars of The Endocrine Society

The Council of The Endocrine Society has established a category of Scholars. These grants have been made available through the generosity of the Schering Corporation and the Upjohn Company, and will be awarded to established investigators and teachers in the field of endocrinology who wish to extend their opportunities for work either in this country or abroad.

The award will not exceed $2,500 annually for each individual and shall be granted on the basis of proposals submitted by the applicant. Such applications should include the estimated financial needs. The funds may be used for travel, maintenance and other expenses.

Nominations

Nominations for the Medal of the Endocrine Society; the Ciba Award; the Ayerst and the Squibb Fellowships may be made by any member of The Endocrine Society. They should be submitted on forms which may be obtained from the Office of the Secretary, 1200 North Walker Street, Oklahoma City 3, Oklahoma. Completed nominations should be returned to the Secretary not later than *November 1* each year.

Proposals for appointment as Scholars of The Endocrine Society should be made in writing by the individual, and addressed to the Secretary of the Society. They should be submitted by *November 1, 1956.*

The Awards Committee will meet in November and notice of awards to successful nominees and applicants will be made not later than December 1.

AUTHOR INDEX, VOLUME 60

ALLEN, J. C., see HOWARD, EVELYN, 785

ARGYRIS, BERTIE F., see GARDNER, W. U., 532

ATERMAN, K. The Thyroid-Adrenal Relationship: The Effect of Cortisone and of Thyroid Hormone on Hepatic Necrosis of Dietary Origin in the Rat, 711

AUSTIN, JANE, see KOCHAKIAN, CHARLES D., 80

AUSTIN, JANE, see KOCHAKIAN, CHARLES D., 144

BACHEM, ERICA, see SREEBNY, LEO M., 200

BAKHTIAR, ABOLFATH KHAN, see BISCHOFF, FRITZ, 333

BARNES, C. M., D. E. WARNER, S. MARKS AND L. K. BUSTAD. Thyroid Function in Fetal Sheep, 325

BARNES, C. M., see GORBMAN, AUBREY, 565

BARRY, JEANNE Q., see FERGUSON, FREDERICK P., 761

BATES, ROBERT W. AND JEROME CORNFIELD. An Improved Assay Method for Thyrotrophin Using Depletion of I^{131} from the Thyroid of Day-Old Chicks, 225

BATES, FRANK, see FRIEDEN, EDWARD H., 270

BATES, FRANK, see FRIEDEN, EDWARD H., 290

BAUMAN, J. W., R. HILL AND I. L. CHAIKOFF. Hepatic Lipogenesis in the Diabetic-Hypophysectomized (Houssay) Rat, 514

BELL, MURIEL E., see GRIESBACH, W. E., 729

BERBER, G. WINSTON, see EHRENSTEIN, MAXIMILIAN, 681

BERGMANN, FRIDA, see RABASA, SOL L., 597

BERNSTEIN, DONALD E. AND GERSON R. BISKIND. Autotransplantation of the Adrenal of the Rat to the Portal Circulation: Induced Adrenal Hypertrophy and Its Prevention by Oophorectomy, 575

BERNSTORF, E. C. Difference in Response of the Capon Comb to Intraperitoneally and Subcutaneously Injected Testosterone Propionate, 173

BEST, MAURICE M., see DUNCAN, CHARLES H., 161

BEYLER, ARTHUR L. AND GORDON O. POTTS. The Effects of Ethinylandrostenediol 3-Cyclohexylpropionate on the Reproductive Tract of Male and Female Rats, 519

BISCHOFF, FRITZ AND ABOLFATH KHAN BAKHTIAR. Delayed Resorption of Various Forms of Insulin, 333

BISKIND, GERSON R., see BERSTEIN, DONALD E., 575

BOGDANOVE, E. M. Selectivity of the Effects of Hypothalamic Lesions on Pituitary Trophic Hormone Secretion in the Rat, 689

BOIS, PIERRE, see SELYE, HANS, 507

BOLKER, H. I., R. D. H. HEARD AND V. J. O'DONNELL. The Biosynthesis of Allopregnane-3B, 16α,20β-Triol from Acetate-1-C^{14} in the Pregnant Mare, 214

BRADBURY, JAMES T. AND THOMAS H. KENT. Protein Hindrance of Pregnanediol Recovery, 162

BRADY, JOSEPH V., see MASON, JOHN W., 741

BRANNICK, L. J., see SWINGLE, W. W., 658

BRINGMAN, E. W., see ZARROW, M. X., 573

BUSTAD, L. K., see BARNES, C. M., 325

BUSTAD, L. K., see GORBMAN, AUBREY, 565

CAHILL, GEORGE F., JR., SYLVIA ZOTTU AND A. SCOTT EARLE. *In Vivo* Effects of Glucagon on Hepatic Glycogen, Phosphorylase and Glucose-6-Phosphatase, 265

CAHILL, JOAN, see CHAPPEL, CLIFFORD I., 677

CALHOUN, D. W., see HERSHBERGER, L. G., 153
CASPI, E., see HECHTER, O., 705
CHAIKOFF, I. L., see BAUMAN, J. W., 514
CHAPPEL, CLIFFORD I., MARIE-PAULE CHAREST, JOAN CAHILL AND GORDON A. GRANT. Reversal of Adrenal Regeneration Hypertension by Amphenone, 677
CHAREST, MARIE-PAUL, see CHAPPEL, CLIFFORD I., 677
CHEEK, WILLIAM R., see GUILLEMIN, ROGER, 488
COLE, B. THEODORE, see ENGLE, FRANK L., 446
COLE, D. F. Effect of Aldosterone on Renal Excretion of Intravenously Administered Saline, 562
CONTOPOULOS, ALEX N., see EVANS, EDWARD S., 403
CORNFIELD, JEROME, see BATES, ROBERT W., 225
COUCH, J. R., see FERGUSON, T. M., 13
COUCH, J. R., see FERGUSON, T. M., 28
COX, ROBERT W., ELAINE D. HENLEY, HIROMICHI T. NARAHARA, PAUL P. VANARSDEL, JR. AND ROBERT H. WILLIAMS. Studies on the Metabolism of Glucagon I^{131} in Rats, 277

DENNIS, B., see FERGUSON, T. M., 28
DEVENUTO, FRANK, see FIRSCHEIN, HILLIARD E., 347
DICZFALUSY, E., A.-M. MAGNUSSON, L. NILSSON and A. WESTMAN. A Method of Bioassay Based on the Duration of Action of Estrogens, 581
DIENGOTT, DANIEL, see MIRSKY, I. ARTHUR, 303
DORFMAN, ALBERT, see SCHILLER, SARA, 376
DUNCAN, CHARLES H., MAURICE M. BEST AND EARLE VAN HEYNINGEN. Qualitative Differences in the Physiologic Activity of Thyroxine and Its Formic Acid Analogue, 161
DYRENFURTH, I. AND E. H. VENNING. Preparation of Urinary Extracts for the Aldosterone Assay: Hydrolysis and Extraction, 136

EARLE, A. SCOTT, see CAHILL, GEORGE F., JR., 265
EHRENSTEIN, MAXIMILIAN, G. WINSTON BERBER AND ROY HERTZ. Progestational Activity of Various Stereoisomers of 19-Nor-Progesterone, 681
EISENSTEIN, ALBERT B. Pantothenic Acid and Adrenocortical Hormone Secretion, 298
EISENSTEIN, ALBERT B. AND PHYLLIS MERRITT HARTROFT. Alterations in the Rat Adrenal Cortex Induced by Sodium Deficiency: Steroid Hormone Secretion, 634
EISENSTEIN, ALBERT B., see HARTROFT, PHYLLIS MERRITT, 641
ELY, CHARLES A. Effect of Antigonadotrophic Serum on Postirradiation Changes in the Reproductive System of Female Mice, 718
EMERSON, J. D. AND G. M. EMERSON. Effect of Steroid Dose and Steroid Ratio on Plateauing in the Adrenalectomized Rat Receiving a Growth Hormone-Containing Pituitary Extract, 393
EMERSON, G. M., see EMERSON, J. D., 393
ENDAHL, GERALD, see KOCHAKIAN, CHARLES D., 80
ENGEL, FRANK L., JOAN FREDERICKS AND B. THEODORE COLE. Sodium Fluoroacetate Diabetes: Insulin and Carbohydrate Tolerance and the Antiketogenic Effects of Glucose and Fructose, 446
ENGEL, FRANK L., see OWEN, JOHN A., JR., 698
ERÄNKÖ, OLAVI AND LIISA RÄISÄNEN. Adrenaline and Noradrenaline in the Adrenal Medulla During Postnatal Development of the Rat, 753
EVANS, EDWARD S., ALEX N. CONTOPOULOS AND MIRIAM E. SIMPSON. Hormonal Factors Influencing Calorigenesis, 403

FERGUSON, T. M., R. H. RIGDON AND J. R. COUCH. Thyroid in B-12 Deficient Chick Embryos, 13
FERGUSON, T. M., J. B. TRUNNELL, B. DENNIS, P. WADE AND J. R. COUCH. The Influence of Vitamin B_{12} Deficiency on the Uptake of I^{131} by the Thyroid Gland in Adult and Embryonic Chickens, 28
FERGUSON, FREDERICK P., DIETRICHE C. SMITH AND JEANNE Q. BARRY. Hypokalemia in Adrenalectomized Dogs During Acute Decompression Stress, 761
FILLIOS, LOUIS. The Gonadal Regulation of Cholesteremia in the Rat, 22
FIRSCHEIN, HILLIARD E., FRANK DEVE-

NUTO, WALTER M. FITCH, ELI M. PEARCE AND ULRICH WESTPHAL. Distribution of Injected Cortisol-4-C^{14} in Normal and Shocked Rats, 347

FITCH, WALTER M., see FIRSCHEIN, HILLIARD E., 347

FLORSHEIM, WARNER, H., NATHAN MOSKOWITZ, JOSEPH R. SCHWARTZ AND M. E. MORTON. A New Assay for Thyrotropic Hormone Based upon Thyroidal Phospholipid Turnover *in vitro*, 683

FORBES, THOMAS R. Plasma Progestin Levels in Progesterone "Anesthesia," 164

FRANK E., see HECHTER, O., 705

FRANK, H., see HECHTER, O., 705

FRANZ, JOHN M. AND GENE F. LATA. Hormonal Effects on Sulfanilamide Acetylation, 602

FREDERICKS, JOAN, see ENGLE, FRANK L., 446

FRIEDEN, EDWARD H. AND FRANK BATES. Effect of Estrogen and Androgen upon Uptake of Glycine-C^{14} by Rat Uteri *In Vitro:* Lack of Concentration Dependence, 270

FRIEDEN, EDWARD H., MORTON R. LABY, FRANK BATES AND NOEL W. LAYMAN. The Effect of Testosterone Propionate upon Incorporation of Labeled Glycine into Mouse Kidney Slices, 290

FRITZ, I. B., M. WEINSTEIN, J. V. MORTON AND R. LEVINE. The Effects of Carbutamide (BZ-55) on Blood Sugar Levels of Depancreatized Dogs Given Insulin, 76

GARDNER, W. U. AND BERTIE F. ARGYRIS. Differences in Vaginal Sensitivity of Mice of Inbred Strains; Localization in the Vagina, 532

GILBERT, CHRISTINE AND JOSEPH GILLMAN. The Importance of Cortisone in Promoting Estrogen-withdrawal Bleeding in the Hypophysectomized Baboon, 570

GILLMAN, JOSEPH, see GILBERT, CHRISTINE, 570

GITLIN, GERSHON. Failure to Detect Squamous Metaplasia in Extra-Genital Organs of the Female Rat Following Prolonged Estrogen Administration, 571

GLICK, JOHN H., JR. The Isolation of Two Corticosteroids from Cattle Bile, 368

GOLDBERG, R. C., J. WOLFF AND R. O. GREEP. Studies on the Nature of the Thyroid-Pituitary Interrelationship, 38

GORBMAN, AUBREY, ALLYN WATERMAN, C. M. BARNES AND L. K. BUSTAD. Thyroidal Function in Fetal and Pregnant Sheep Given Chronic Low Level Dosages of I^{131}, 565

GOY, R. W., see HOAR, R. M., 337

GRANT, GORDON A., see CHAPPEL, CLIFFORD I., 677

GREEP, R. O., see GOLDBERG, R. C., 38

GREEP, ROY O., see KNOBIL, ERNEST, 166

GRIESBACH, W. E., MURIEL E. BELL AND MURIEL LIVINGSTON. Changes in the Pituitary Gonadotrophs of the Vitamin E-Deficient Male Rat, 729

GUILLEMIN, ROGER, WALTER R. HEARN, WILLIAM R. CHEEK AND DWIGHT E. HOUSHOLDER. Control of Corticotrophin Release: Further Studies with *In Vitro* Methods, 488

HAGAN, SUSIE N., see SCOW, ROBERT O., 273

HAGERMAN, DWAIN D., see VILLEE, CLAUDE A., 552

HARDY, JR., L. B., see MELLEN, W. J., 547

HARKIN, JAMES C. An Electron Microscopic Study of the Castration Changes in the Rat Prostate, 185

HARTROFT, PHYLLIS MERRITT, see EISENSTEIN, ALBERT B., 634

HARTROFT, PHYLLIS MERRITT AND ALBERT B. EISENSTEIN. Alterations in the Adrenal Cortex of the Rat Induced by Sodium Deficiency: Correlation of Histologic Changes with Steroid Hormone Secretion, 641

HARWOOD, C. THERESA AND JOHN W. MASON. Acute Effects of Tranquilizing Drugs on the Anterior Pituitary-ACTH Mechanism, 239

HAWKER, R. W. AND P. A. ROBERTSON. Oxytocin in Human Female Blood, 652

HEARD, R. D. H., see BOLKER, H. I., 214

HEARN, WALTER R., see GUILLEMIN, ROGER, 488

HECHTER, O., E. FRANK, E. CASPI AND H. FRANK. Corticosteroid Metabolism in Liver: *In Vivo* Metabolism of Cortisone and Cortisol by Dog Liver, 705

HENLEY, ELAINE D., see COX, ROBERT O., 277

HERSHBERGER, L. G. AND D. W. CALHOUN. A Cotton Pellet Granuloma Assay for Locally Administered Anti-Inflammatory Drugs: 9 Halo, 21 Desoxy-Corticoids, 153

HERSHBERGER, LEE G., see MEYER, ROLAND K., 397

HERTZ, ROY, see EHRENSTEIN, MAXIMILIAN, 681

HILL, R., see BAUMAN, J. W., 514

HILLIARD, JESSAMINE AND PHILIP M. WEST. Pepsin Inhibitor in Pituitary Extracts, 797

HOAR, R. M., R. W. GOY AND W. C. YOUNG. Loci of Action of Thyroid Hormone on Reproduction in the Female Guinea Pig, 337

HOFMANN, FREDERICK G. The *In Vitro* Hydroxylation of 21-Carbon Steroids by Rat Adrenal Glands, 382

HOUSEHOLDER, DWIGHT E., see GUILLEMIN, ROGER, 488

HOWARD, EVELYN AND J. C. ALLEN. The Retarding Influences of Adrenalectomy and Age on Estradiol Induced Connective Tissue Hyperplasia in Seminal Vesicles, 785

HUNSAKER, WALTER, see POLIN, DONALD, 1

JESSUP, D. C., N. B. G. TAYLOR AND R. L. NOBLE. A Comparison of Methods for Extracting Pituitary Antidiuretic Substance from Urine, 6

JINKS, ROBERT, see MIRSKY, I. ARTHUR, 318

JØRGENSEN, C. BARKER AND PER ROSENKILDE. Chloride Balance in Hypophysectomized Frogs, 219

KENNY, ALEXANDER D. Suppression of Calcium-Mobilizing Activity of Parathyroid Extracts by Inorganic Phosphate, 423

KENT, THOMAS H., see BRADBURY, JAMES T., 162

KNOBIL, ERNEST, RICHARD C. WOLF, ROY O. GREEP AND ALFRED E. WILHELMI. Effect of a Primate Pituitary Growth Hormone Preparation on Nitrogen Metabolism in the Hypophysectomized Rhesus Monkey, 166

KNOWLTON, ABBIE, I., EMILY N. LOEB AND HERBERT C. STOERK. Effect of Synthetic Analogues of Hydrocortisone on the Blood Pressure of Adrenalectomized Rats on Sodium Restriction, 768

KOCHAKIAN, CHARLES D., GERALD ENDAHL AND JANE AUSTIN. The Influence of Testosterone on the Growth of the Urinary Bladder, 80

KOCHAKIAN, CHARLES D., CAROL TILLOTSON AND JANE AUSTIN. A Comparison of the Effect of Inanition, Castration and Testosterone of the Muscles of the Male Guinea Pig, 144

KOCHAKIAN, CHARLES D. AND CAROL TILLOTSON. Influence of Several C_{19} Steroids on the Growth of Individual Muscles of the Guinea Pig, 607

KOSTYO, JACK L. A Study of the Glycogen Levels of the Rat Uterus and Certain Skeletal Muscles During Pregnancy, 33

KRAMÁR, JENÖ, V. WILLIAM MEYERS, HARRY H. MCCARTHY AND MARGARET SIMAY-KRAMÁR. Further Study on the Endocrine Relations of Capillary Resistance, 589

KRAMÁR-SIMAY, MARGARET, see KRAMÁR, JENÖ, 589

LABY, MORTON R., see FRIEDEN, EDWARD H., 290

LANGFORD, H. G. Production of Exophthalmos in *Fundulus heteroclitus* (Var. Bermudae) by Triiodothyronine and Desiccated Thyroid, 390

LANMAN, JONATHAN T. AND LILLIAN MOSHEL SILVERMAN. *In Vitro* Steroidogenesis in the Human Neonatal Adrenal Gland, Including Observations on Human Adult and Monkey Adrenal Glands, 433

LATA, GENE F., see FRANZ, JOHN M., 602

LAYMAN, NOEL W., see FRIEDEN, EDWARD H., 290

LEONARD, SAMUEL L. The Effect of Hormones on Phosphorylase Activity in Skeletal Muscle, 619

LEVEY, HAROLD A. AND DAVID H. SOLOMON. Studies on the Metabolism of Thyrotropin, 118

LEVINE, R., see FRITZ, I. B., 76

LI, CHOH HAO, see LOSTROH, ARDIS J., 308

LIVINGSTON, MURIEL, see GRIESBACH, W. E., 729

LOEB, EMILY N., see KNOWLTON, ABBIE I., 768

LOSTROH, ARDIS, J. AND CHOH HAO LI.

Bioassay of Hypophyseal Growth Hormone in Hypophysectomized Mice by the Tibia Test, 308
LUKENS, F. D. W., see OWEN-VALLANCE, 625

MACFARLANE, W. V., see ROBINSON, KATHLEEN W., 679
MAGNUSSON, A.-M., see DICZFALUSY, E., 581
MARKS, S., see BARNES, C. M., 325
MASON, JOHN W., see HARWOOD, C. THERESA, 239
MASON, JOHN W., JOSEPH V. BRADY AND MURRAY SIDMAN. Plasma 17-Hydroxycorticosteroid Levels and Conditioned Behavior in the Rhesus Monkey, 741
MCCANN, S. M. The ACTH-Releasing Activity of Extracts of the Posterior Lobe of the Pituitary *In Vivo*, 664
MCCARTHY, HARRY H., see KRAMÁR, JENÖ, 589
MCKERNS, KENNETH W. The Effect of Some Natural and Synthetic Sex Hormones on Adrenal Cortical Secretion *In Vitro*, 130
MELLEN, W. J. AND L. B. HARDY, JR. Blood Protein-Bound Iodine in the Fowl, 547
MEYER, JULIA, see SREEBNY, LEO M., 200
MEYER, ROLAND K. AND LEE G. HERSHBERGER. Effects of Testosterone Administration on Acid Soluble and Insoluble Glycogen in the Levator Ani Muscle, 397
MEYERS, V. WILLIAM, see KRAMÁR, JENÖ, 589
MIRSKY, I. ARTHUR, GLADYS PERISUTTI AND DANIEL DIENGOTT. Effect of Fasting on Insulinase Activity and on Hypoglycemic Response to Insulin, 303
MIRSKY, I, ARTHUR, GLADYS PERISUTTI AND ROBERT JINKS. The Hypoglycemic Action of Metabolic Derivatives of L-Tryptophan by Mouth, 318
MORTON, J. V., see FRITZ, I. B., 76
MORTON, M. E., see FLORSHEIM, WARNER H., 683
MOSIER, H. DAVID. Comparative Histological Study of the Adrenal Cortex of the Wild and Domesticated Norway Rat, 460
MOSKOWITZ, NATHAN, see FLORSHEIM, WARNER H., 683

NARAHARA, HIROMICHI T., see COX, ROBERT W., 277
NARAHARA, H. T., AND ROBERT H. WILLIAMS. Degradation of Glucagon-I^{131} by Rat Tissues *In Vitro*, 285
NELSON, WARREN O., see STEINBERGER, E., 105
NEW BOOK REGISTER, 329
NILSSON, L., see DICZFALUSY, E., 581
NOBLE, R. L., see JESSUP, D. C., 6

O'DONNELL, V. J., see BOLKER, H. I., 214
OWEN, JOHN A., JR., AND FRANK L. ENGEL. Temporary Growth Hormone Diabetes in the Tube-Fed Rat, 698

PARLOW, A. F., see SWINGLE, W. W., 658
PAULY, JOHN E. Morphological Observations on the Adrenal Cortex of the Laboratory Rat, 247
PEARCE, ELI M., see FIRSCHEIN, HILLIARD E., 347
PERISUTTI, GLADYS, see MIRSKY, I. ARTHUR, 303
PERISUTTI, GLADYS, see MIRSKY, I. ARTHUR, 318
POLIN, DONALD, PAUL D. STURKIE AND WALTER HUNSAKER. The Blood Calcium Response of the Chicken to Parathyroid Extracts, 1
POLIN, D. AND P. D. STURKIE. The Influence of the Parathyroids on Blood Calcium Levels and Shell Deposition in Laying Hens, 778
POSTEL, SHOLEM. Placental Transfer of Perchlorate and Triiodothyronine in the Guinea Pig, 53
POTTS, GORDON O., see BEYLER, ARTHUR L., 519
PRUITT, RALPH L., see RICHARDS, JOHN B., 99

RABASA, SOL L. AND FRIDA BERGMANN. A Method to Reduce the Individual Variation in an Antidiuretic Assay, 597
RÄISÄNEN, LIISA, see ERÄNKÖ, OLAVI, 753
RALL, J. E., see TATA, JAMSHED R., 83
RAWSON, RULON W., see TATA, JAMSHED R., 83
REICHLIN, SEYMOUR. The Effect of Dehydration, Starvation, and Pitressin Injections on Thyroid Activity in the Rat, 470
REICHLIN, SEYMOUR. The Effect of Hypothalamic Lesions upon the Thyroid

Response to Partial Thyroidectomy, 567
RICHARDS, JOHN B. AND RALPH L. PRUITT. Hydrocortisone Suppression of Stress-Induced Adrenal 17-Hydroxycorticosteroid Secretion in Dogs, 99
RIGDON, R. H., see FERGUSON, T. M., 13
RINFRET, A. P., see ROBERTSON, O. H., 559
ROBERTSON, O. H. AND A. P. RINFRET. Maturation of the Infantile Testes in Rainbow Trout (*Salmo gairnerii*) Produced by Salmon Pituitary Gonadotrophins Administered in Cholesterol Pellets, 559
ROBERTSON, P. A., see HAWKER, R. W., 652
ROBINSON, KATHLEEN W. AND W. V. MACFARLANE. Plasma Antidiuretic Activity of Marsupials During Exposure to Heat, 679
ROSENKILDE, PER, see JØRGENSEN, C. BARKER, 219

SELYE, HANS AND PIERRE BOIS. Effect of Corticoids upon Experimental Lathyrism, 507
SCHILLER, SARA AND ALBERT DORFMAN. The Metabolism of Mucopolysaccharides in Animals: The Effect of Cortisone and Hydrocortisone on Rat Skin, 376
SCHWARTZ, JOSEPH R., see FLORSHEIM, WARNER H., 683
SCOW, ROBERT O. AND SUSIE N. HAGAN. Effect of Testosterone Propionate on Myosin, Collagen and Other Protein Fractions in Striated Muscle of Gonadectomized Rats, 273
SCOW, ROBERT O. "Total" Pancreatectomy in the Rat: Operation, Effects, and Postoperative Care, 359
SHELESNYAK, M. C. Gonadotrophic Content of Pituitary of Pregnant and Pseudopregnant Rats Following Single Injection of Ergotoxine, 802
SIDMAN, MURRAY, see MASON, JOHN W., 741
SILBERBERG, MARTIN, see RUTH SILBERBERG, 67
SILBERBERG, RUTH AND MARTIN SILBERBERG. Changes in Bones and Joints of Underfed Mice Bearing Anterior Hypophyseal Grafts, 67
SILVERMAN, LILLIAN MOSHEL, see LANMAN, JOHATHAN T., 433
SIMPSON, MIRIAM E., see EVANS, EDWARD S., 403
SINGER, B. Aldosterone in the Adrenal Vein Blood of Nephrotic Rats, 420
SMITH, DIETRICHE C., see FERGUSON, FREDERICK P., 761
SOLOMON, DAVID H., see LEVEY, HAROLD A., 118
SREEBNY, LEO M., JULIA MEYER, ERICA BACHEM AND J. P. WEINMANN. Restoration of Enzymatic Activity in the Submaxillary Gland of the Hypophysectomized Albino Rat, 200
STEINBERGER, E., AND WARREN O. NELSON. The Effect of Furadroxyl Treatment and X-Irradiation on the Hyaluronidase Concentration of Rat Testes, 105
STOERK, HERBERT C., see KNOWLTON, ABBIE I., 768
STURKIE, PAUL D., see POLIN, DONALD, 1
STURKIE, P. D., see POLIN, D., 778
SWANSON, HEIDI EARTLY. The Effect of Temperature on the Potentiation of Adrenalin by Thyroxine in the Albino Rat, 205
SWINGLE, W. W., L. J. BRANNICK AND A. F. PARLOWL. High Potency of 2-Methyl-9α-Fluorohydrocortisone for Life Maintenance and Revival from Insufficiency of Adrenalectomized Dogs, 658

TATA, JAMSHED R., J. E. RALL AND RULON W. RAWSON. Metabolism of L-thyroxine and L-3:5:3′-triiodothyronine by Brain Tissue Preparations, 83
TAYLOR, N. B. G., see JESSUP, D. C., 6
TILLOTSON, CAROL, see KOCHAKIAN, CHARLES D., 144
TILLOTSON, CAROL, see KOCHAKIAN, CHARLES D., 607
TRUNNELL, J. B., see FERGUSON, T. M., 28

VALLANCE-OWEN, J. AND F. D. W. LUKENS. Studies on Insulin Antagonism in Plasma, 625
VANARSDEL, PAUL, JR., see COX, ROBERT W., 277
VAN HEYNINGEN, EARLE, see DUNCAN, CHARLES H., 161
VENNING, E. H., see DYRENFURTH, I., 136
VILLEE, CLAUDE A. AND DWAIN D. HAGERMAN. Compounds with Antiestrogenic Activity *In Vitro*, 552

WADE, P., see FERGUSON, T. M., 28
WARNER, D. E., see BARNES, C. M., 325
WATERMAN, ALLYN, see GORBMAN, AUBREY, 565
WEINMANN, J. P., see SREEBNY, LEO M., 200
WEINSTEIN, M., see FRITZ, I. B., 76
WEST, PHILIP M., see HILLIARD, JESSAMINE, 797
WESTMAN, A., see DICZFALUSY, E., 581
WESTPHAL, ULRICH, see FIRSCHEIN, HILLIARD E., 347
WILHELMI, ALFRED E., see KNOBIL, ERNEST, 166
WILLIAMS, ROBERT H., see COX, ROBERT W., 277
WILLIAMS, ROBERT H., see NARAHARA, H. T., 285
WOLF, RICHARD C., see KNOBIL, ERNEST, 166
WOLFF, J., see GOLDBERG, R. C., 38

YOUNG, W. C., see HOAR, R. M., 337

ZARROW, M. X. AND E. W. BRINGMAN. Decreased Sensitivity of the Eosinophil Depletion Test in the Rat Exposed to a Low Ambient Temperature, 573
ZOTTU, SYLVIA, see CAHILL, GEORGE F., 265

SUBJECT INDEX, VOLUME 60

Adrenal cortex
- hydrocortisone suppression of stress-induced adrenal 17-OH-CS secretion in dogs, 99
- secretion *in vitro* as influenced by sex steroids, 130
- anti-inflammatory action and cotton pellet granuloma assay, 153
- study of morphology in rat, 247
- secretion as influenced by pantothenic acid, 298
- distribution of cortisol-4-C^{14} in normal and shocked rats, 347
- isolation of two corticoids from bile, 368
- effects of corticoids on mucopolysaccharides in rat skin, 376
- *in vitro* hydroxylation of 21-carbon steroids, 382
- influence on calorigenesis, 403
- *in vitro* steroidogenesis by human and monkey adrenal glands, 433
- histology in wild and domesticated rats, 460
- corticoid effect on lathyrism, 507
- aldosterone effect on excretion of i.v. saline, 562
- in estrogen withdrawal bleeding in hypophysectomized baboon, 570
- function after autotransplantation into portal circulation, 575
- relations to capillary resistance, 589
- influence on phosphorylase activity in skeletal muscle, 619
- alterations in function induced by sodium deficiency, 634
- alterations in structure and function induced by sodium deficiency, 641
- insufficiency symptoms and revival by 2-methyl-9α-fluorohydrocortisone in dogs, 658
- regeneration hypertension reversed by amphenone, 677
- effect of cortisone on hepatic necrosis of dietary origin in the rat, 711
- function and conditioned behavior in rhesus monkey, 741
- and hypokalemia in acute decompression stress, 761
- ablation and effect of analogues of hydrocortisone on blood pressure during sodium restriction, 768
- ablation and age retard estrogen induced connective tissue hyperplasia in seminal vesicles, 785

Adrenal medulla
- temperature effect on potentiation of adrenalin by thyroxine, 205
- and phosphorylase activity in skeletal muscle, 619
- during postnatal development, 753

Aldosterone
- preparation of urine extracts for assay, 136
- in adrenal vein blood of nephrotic rats, 420
- effect on renal excretion of saline i.v., 562

Androgens
- influence of testosterone on growth of urinary bladder, 80
- influence on guinea pig muscles of inanition and castration, 144
- comparison of routes of injection on capon comb response to testosterone propionate, 173
- testosterone effect on submaxillary gland enzymes after hypophysectomy, 200
- effect on glycine-C^{14} uptake in rat uterus, 270
- action on myosin, collagen, *et cet ra*, in striated muscle of gonadectomized rats, 273
- effect on incorporation of labeled glycine in mouse kidney slices, 290
- effect of T.P. on acid soluble and insoluble glycogen in levator ani, 397
- influence of C-19 steroids on growth of individual muscles in guinea pig, 607

Antigonadotrophin
- effect of antiserum on post-irradiation changes in reproductive system of female mice, 718

Corticotropin
- secretion as influenced by tranquilizing drugs, 239

control of release, an *in vitro* study, 488
releasing activity of extracts of pars neuralis *in vivo*, 664

Eosinophil
decreased sensitivity of depletion test in rat exposed to low ambient temperature, 573
Estrogens
effect on cholesteremia in rat, 22
effect on glycine-C^{14} uptake by rat uterus *in vitro*, 270
and vaginal sensitivity of mice of inbred strains, 532
and compounds with antiestrogenic activity *in vitro*, 552
prolonged action failed to produce squamous metaplasia, 571
bioassay based on duration of action, 581
induced hyperplasia of connective of seminal vesicles in rats as influenced by adrenalectomy, 785

Glucagon
in vivo effect on hepatic glycogen, phosphorylase and glucose-6-phosphatase, 265
metabolism in rats, 277
degradation by rat tissue *in vivo*, 285
Growth hormone
of monkey origin, and N retention in hypophysectomized monkey, 166
bioassay by tibia test in hypophysectomized mice, 308
effect in relation to steroids on plateauing in adrenalectomized rats, 393
temporary diabetes in tube-fed rats, 698

Insulin
delayed absorption of various forms of, 333
and carbohydrate tolerance and the antiketogenic effects of glucose and fructose in sodium fluoroacetate diabetes, 446
antagonism in plasma, 625

Ovary
effect on cholesteremia in rat, 22

Pancreas
the effects of carbutamide on blood sugar level of depancreatized dog given insulin, 76
effect of glucagon on hepatic glycogen and phosphatases, 265
effect of fasting on insulinase activity and hypoglycemic response to insulin, 303
hypoglycemic action of metabolic derivatives of l-tryptophan, 318
"total" ablation: operation, effects and after care, 359
and the antiketogenic effects of glucose and fructose, 446
in relation to hepatic lipogenesis, 514
Parathyroid
blood calcium response in chicken, 1
calcium-mobilizing action suppressed by inorganic phosphate, 423
influence on blood calcium and shell deposition in laying hens, 778
Pituitary gland
nature of interrelationship with thyroid, 38
graft effects on bones and joints in underfed mice, 67
metabolism of thyrotropin, 118
ablation effect on chloride balance in frogs, 219
improved thyrotropin assay, 225
ACTH secretion as influenced by tranquilizing drugs, 239
factors influencing calorigenesis, 403
in relation to hepatic lipogenesis, 516
gonadotropin of fish stimulate trout testes, 559
secretion of TSH as influenced by hypothalamic lesions, 567
in relation to capillary resistance, 589
selective effects of hypothalamic lesions on secretion of trophic hormones, 689
gonadotrops in vitamin E deficient male rat, 729
extracts contain pepsin inhibitor, 797
gonadotrophin content of pregnant and pseudopregnant rats after ergotoxine, 802
antidiuretic substances in human urine, 6
reducing variation in antidiuretic assay, 597
oxytocin in human female blood, 652
pars neuralis extracts release ACTH *in vivo*, 664
antidiuretic activity in plasma of marsupials exposed to heat, 679
Placenta
transfer of perchlorate and triiodothyronine in the guinea pig, 53
Progesterone
"anesthesia," and plasma levels, 164

-like activity of stereoisomers of 19-nor-progesterone, 681

Steroids
anti-inflammatory action with local application, 153
protein hindrance of pregnanediol recovery, 162
biosynthesis of allopregnane-3β, 16α, 20β-triol from acetate-1-C^{14} in the pregnant mare, 214
isolation of two corticoids from cattle bile, 368
hydroxylation *in vitro* of 21-C steroids by rat adrenal, 382
dose and ratio on plateauing in adrenalectomized rats given growth hormone, 393
formation *in vitro* by human and monkey adrenals, 433
effects of ethinylandrostenediol 3-cyclohexylpropionate on the reproductive tracts in rats, 519
in relation to acetylation of sulfanilimide, 602
2-methyl-9-fluorhydrocortisone in maintenance of adrenalectomized dogs, 658
progestational activity of various stereoisomers of 19-nor-progesterone, 681
in vivo metabolism of corticoids in perfused dog livers, 705
effect of analogues of hydrocortisone on blood pressure in rats, 768

Testes
effect on cholesteremia in rat, 22
hyaluronidase concentration as influenced by furadroxyl and x-irradiation, 105
castration changes in rat prostate by electron microscopy, 185
maturation in trout given fish gonadotropin, 559

Thyroid gland
effect in B-12 deficient chick embryos, 13
uptake of I^{131} as influenced by B_{12} deficiency in adult and embryonic chickens, 28
nature of interrelationship with pituitary, 38
placental transfer of perchlorate and triiodothyronine in the guinea pig, 53
effect of brain tissue on metabolism of L-thyroxine and L-3:5:3′-triiodothyronine, 83
physiologic activity of thyroxine and a formic acid analogue, 161
effect on salivary enzymes after hypophysectomy, 200
thyroxine potentiation of adrenalin as influenced by temperature, 205
function in fetal sheep, 325
hormone loci of action on reproduction in the female guinea pig, 337
exophthalmus in Fundulus as produced by triiodothyronine and desiccated thyroid, 390
activity as influenced by dehydration, starvation, and pitressin injections, 470
and blood protein-bound iodine in fowl, 547
fetal and maternal function in sheep on low I^{131} intake, 565
effect on hepatic necrosis of dietary origin, 711

Thyrotropin
metabolism of, 118
an improved assay method, I^{131} depletion in day-old chick, 225
secretion after thyroidectomy, as influenced by hypothalamic lesions, 567
a new assay based on thyroidal phospholipid turnover *in vitro*, 683

Uterus
glycogen levels of skeletal muscles during pregnancy, 33
glycine-C^{14} uptake *in vitro* as influenced by sex hormones, 270
importance of cortisone in promoting estrogen-withdrawal bleeding in hypophysectomized baboon, 570

Vol

E

Ar

DOES NOT CIRCULATE
JUN 17 1957
HOSPITAL LIBRARY

Volume 60 JUNE, 1957 Number 6

ENDOCRINOLOGY

FLORSHEIM, WARNER H., NATHAN MOSKOWITZ, JOSEPH R. SCHWARTZ AND M. E. MORTON
A New Assay for Thyrotropic Hormone Based upon Thyroidal Phospholipid Turnover *in vitro* 683
BOGDANOVE, E. M.
Selectivity of the Effects of Hypothalamic Lesions on Pituitary Trophic Hormone Secretion in the Rat 689
OWEN, JOHN A., JR. AND FRANK L. ENGEL
Temporary Growth Hormone Diabetes in the Tube-Fed Rat 698
HECHTER, O., E. FRANK, E. CASPI AND H. FRANK
Corticosteroid Metabolism in Liver: *In Vivo* Metabolism of Cortisone and Cortisol by Dog Liver 705
ATERMAN, K
The Thyroid-Adrenal Relationship: The Effect of Cortisone and of Thyroid Hormone on Hepatic Necrosis of Dietary Origin in the Rat 711
ELY, CHARLES A.
Effect of Antigonadotrophic Serum on Post-irradiation Changes in the Reproductive System of Female Mice 718
GRIESBACH, W. E., MURIEL E. BELL AND MURIEL LIVINGSTON
Changes in the Pituitary Gonadotrophs of the Vitamin E-Deficient Male Rat 729
MASON, JOHN W., JOSEPH V. BRADY AND MURRAY SIDMAN
Plasma 17-Hydroxycorticosteroid Levels and Conditioned Behavior in the Rhesus Monkey 741
ERÄNKÖ, OLAVI AND LIISA RÄISÄNEN
Adrenaline and Noradrenaline in the Adrenal Medulla During Postnata Development of the Rat 753
FERGUSON, FREDERICK P., DIETRICH C. SMITH AND JEANNE Q. BARRY
Hypokalemia in Adrenalectomized Dogs During Acute Decompression Stress 761
KNOWLTON, ABBIE I. EMILY N LOEB AND HERBERT C. STOERK
Effect of Synthetic Analogues of Hydrocortisone on the Blood Pressure of Adrenalectomized Rats on Sodium Restriction 768
POLIN, D. AND P. D. STURKIE
The Influence of the Parathyroids on Blood Calcium Levels and Shell Deposition in Laying Hens 778
HOWARD, EVELYN AND J. C. ALLEN
The Retarding Influences of Adrenalectomy and Age on Estradiol-Induced Connective Tissue Hyperplasia in Seminal Vesicles 785
HILLIARD, JESSAMINE AND PHILIP M. WEST
Pepsin Inhibitor in Pituitary Extracts 797
NOTES AND COMMENTS
SHELESNYAK, M. C.
Gonadotrophin Content of Pituitary of Pregnant and Pseudopregnant Rats Following Single Injection of Ergotoxine 802
SAUNDERS, FRANCIS J., RICHARD A. EDGREN AND VICTOR A. DRILL
On the Progestational Activity of 17α-Ethynyl-17-hydroxy-5(10)-estren-3-one (Norethynodrel) 804
BOOK REVIEW 806
THE ENDOCRINE SOCIETY 811
INDEX 813

ISSUED MONTHLY FOR THE ENDOCRINE SOCIETY
BY CHARLES C THOMAS, PUBLISHER,
SPRINGFIELD, ILLINOIS

Annual Subscription $15.00 Single Copies $1.50

"A. P. L." therapy in cryptorchidism is now considered at an earlier age

PRESENT TREND ALSO FAVORS HIGHER DOSAGES THAN PREVIOUSLY EMPLOYED

There are widely divergent views on the management of cryptorchidism. However, many practitioners now believe that an early descent of the testes is essential if spermatogenesis is to be preserved. Furthermore, higher doses than formerly used are now recommended on the basis that these will produce a greater incidence of satisfactory responses.[1] In a recent issue of the Journal of the American Medical Association, an editorial suggested a therapeutic trial with chorionic gonadotropin when there is evidence of testicular hormone deficiency[2] such as obesity, signs of feminism or enlarged breasts.

In the treatment of cryptorchidism, "A.P.L." can be used...

Diagnostically, because failure to respond to a full course of "A. P. L." therapy may indicate an anatomic obstruction.[1]

Definitively, because in both unilateral and bilateral cryptorchidism a significant percentage of testes will descend following treatment with "A.P.L."

Preoperatively, because "A.P.L." will usually facilitate orchiopexy by lengthening the cords and enlarging the testes.[3]

Postoperatively, because follow-up therapy with "A.P.L." may prevent retraction of the testes.

"A.P.L."®

Brand of chorionic gonadotropin [human]

virtually painless on injection

practically free from local reactions

Suggested Dosage Regimens [depending on age]:

[1] 4,000 I. U., three times weekly, for two to three weeks, or

[2] 1,000 I. U., three times weekly, for six to eight weeks.

Caution: Evidence of sexual precociousness is an indication for the immediate withdrawal of treatment. After regression of undue development, reduced dosage regimen may be resumed.

Availability:

In Dry Form

No. 972—Each package provides one "Secule"® containing 20,000 I. U. and 10 cc. vial of sterile diluent containing 0.5% phenol.

In Sterile Solution

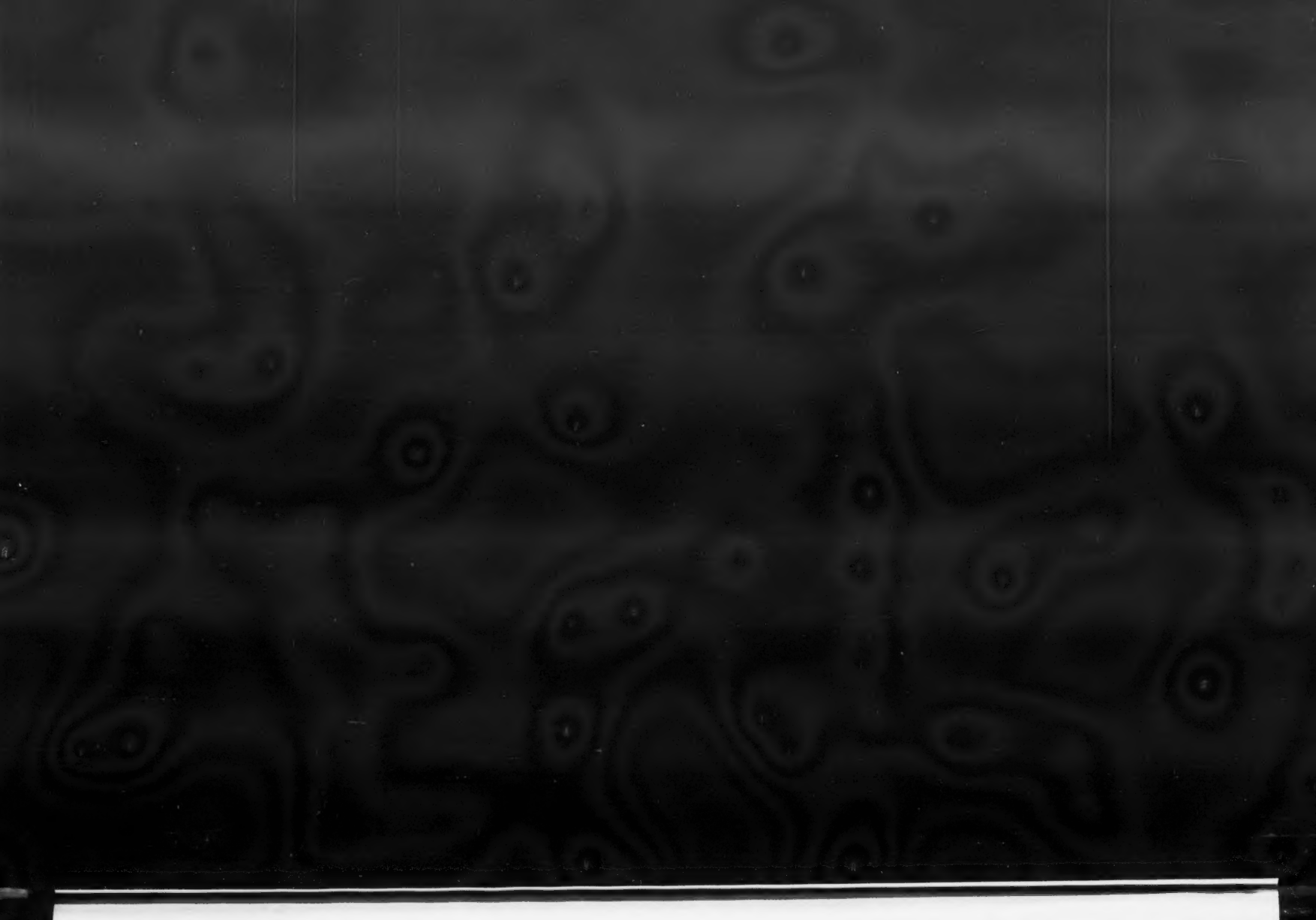
No. 999—1,000 I. U. per cc.—10 cc. vials

No. 500—500 I. U. per cc.—10 cc. vials

Bibliography: 1. Hand, J. R.: J. Urol. 75:973 (June) 1956. • 2. Editorial: J. A. M. A. 163: 650 (Feb. 23) 1957. • 3. Lapin, J. H., Klein, W., and Goldman, A.: J. Pediat. 22:175 (Feb.) 1943.

AYERST LABORATORIES
New York, N.Y. • Montreal, Canada

5716



Antibiotica et Chemotherapia

Advances

Edited by

H. BLOCH, New York O. GSELL, Basel E. LANGER, Berlin

VOL. 1 X + 378 p., 113 fig., 1954. **US $12.50**

VOL. 2 VI + 300 p., 21 fig., 1955. **US $9.60**

VOL. 3 VIII + 398 p., 45 fig., 1956. **US $13.45**

From the Contents of Volumes 1-3

H. SELYE, Montreal: **The Stress-Concept as it Presents Itself in 1956.**

W. P. LONGMIRE, Jr., and S. W. SMITH, Los Angeles, Calif.: **The Surgical Use of Presently Available Antibiotics.**

R. L. MAYER and P. C. EISMAN, Summit, N.J.: **Chemotherapeutic Relationships between Fungi and Mycobacteria.**

P. KALLÓS und L. KALLÓS-DEFFNER, Hälsingborg: **Allergie und Antibiotica.**

P. RENTCHNICK, Genève: **L'évolution clinique et thérapeutique des staphylococcies.**

S. HELANDER, Stockholm: **Sulfonamide Combinations.**

E. ROSSI, Zürich: **Antibiotica in der Pädiatrie.**

P. RENTCHNICK, Genève: **Les accidents provoqués par les antibiotiques.**

T. WEGMANN, Zürich: **Pilzerkrankungen der inneren Organe als Folge von Behandlung mit Antibiotica, unter besonderer Berücksichtigung des Respirationstraktes.**

H. BARTELHEIMER und W. ENGERT, Berlin: **Die Behandlung der Endocarditis lenta mit Antibiotica.**

V. PIRILÄ, Helsinki: **Cutaneous and Serologic Tests in Allergy to Antibiotics.**

H. BEERMAN and J. EDEIKEN, Philadelphia: **The Penicillin Treatment of Cardiovascular Syphilis.**

M. WELSCH, Liège: **La résistance bactérienne aux antibiotiques. Aspects biologiques.**

O. GSELL, Basel: **Klinische Probleme der Antibiotica-Therapie.**

BASEL 11 (Switzerland) S. KARGER NEW YORK

For U.S.A.: Albert J. Phiebig, P.O. Box 352, White Plains, N.Y.

THE ENDOCRINE SOCIETY

LAWSON WILKINS, *President* — ELEANOR VENNING, *Vice-President*
LEO T. SAMUELS, *President-Elect* — HENRY H. TURNER, *Secretary-Treasurer*

COUNCIL: ROY HERTZ, JOSEPH W. JAILER, ELEANOR VENNING, EARL T. ENGLE, LEONARD I. PUGSLEY, ALLAN T. KENYON, WARREN O. NELSON, E. B. ASTWOOD, HENRY H. TURNER, LEO T. SAMUELS, A. ALBERT, LAWSON WILKINS.

PUBLICATION COMMITTEE: F. D. W. LUKENS (*chairman*), E. B. ASTWOOD, RULON RAWSON, E. P. MCCULLAGH, ROY HOSKINS, JOHN EAGER HOWARD, H. H. TURNER.

ENDOCRINOLOGY

Editorial Board

A. ALBERT
F. C. BARTTER
R. GAUNT
R. HERTZ
L. I. PUGSLEY
EVELYN ANDERSON
J. T. BRADBURY
N. S. HALMI
D. J. INGLE
C. H. SAWYER
B. L. BAKER
R. I. DORFMAN
R. D. H. HEARD
W. R. LYONS
A. E. WILHELMI

ROY O. GREEP, *Managing Editor*
25 Shattuck St., Boston 15, Massachusetts

ENDOCRINOLOGY is published monthly by The Endocrine Society. Annual subscription price is as follows: In the United States, U.S. Possessions, Pan-American Union & Spain, $15.00; Canada, $15.50; other foreign countries, $16.00. Single copies, $1.50 postpaid. Distributed in the British Commonwealth of Nations by Blackwell Scientific Publications, Ltd., 24-25 Broad Street, Oxford, England. Subscription price £5.15s.0d. Copyrighted in the British Commonwealth by Blackwell Scientific Publications, Oxford. No part may be duplicated or reproduced without permission of publishers.

INSTRUCTIONS TO AUTHORS

Preference will be given to papers which are contributive to fundamental knowledge of the endocrines. The JOURNAL OF CLINICAL ENDOCRINOLOGY AND METABOLISM, also published by The Endocrine Society, is held to be a more suitable outlet for papers on clinical endocrine medicine and case reports. Papers should be written with the greatest concision compatible with clarity and accuracy. Papers presenting negative or confirmatory results are considered only for the section on NOTES and COMMENTS. Submission of a paper to The Endocrine Society's journals is held to imply that it is not to be published elsewhere. Papers are published in the order of their acceptance with the exception that those given a low priority rating by the Editors may be delayed at the discretion of the Managing Editor.

NOTES and COMMENTS, a section appearing at the end of most issues is designed primarily for the prompt publication of material of unusual interest. Other papers suitable for this section include those in the nature of progress reports, those describing new methods, techniques or apparatus, and those describing results of a confirmatory or negative nature. Manuscripts must not exceed four double-spaced typewritten sheets. Tabular and illustrative material must be kept to a minimum.

NEW BOOKS will be listed as they are received and those which bear on topics related to endocrinology will be reviewed as promptly as possible and as space permits.

Reprints: A price list and order blanks for reprints will be sent with the Galley proofs.

Preparation of Manuscripts: *Specifications:*

1. Double-spaced or triple-spaced typewritten copy should be submitted in duplicate.
2. Tables, references, legends and footnotes should be on separate sheets.
3. In the text indicate citations to literature by number (in parentheses) and list references in numerical order at the end of the paper. Abbreviations of the names of journals should correspond to the system used by the Quarterly Cumulative Index Medicus. References should be typed as follows: Taurog, A., I. L. Chaikoff and D. D. Feller: *J. Biol. Chem.* 171: 189. 1947. The references cited should have an important bearing on the work being reported.
4. The following type of abbreviation should be employed: gm., mg., μg., cc., m., cm., mm., μ, μμ, C., m.eq., R.U., U., I.U.
5. If the proprietary names of substances employed are to be given they should be appropriately identified as such.
6. Use "Fig." in parentheses and at the beginning of a legend; as a part of the text use "Figure," "Table" and "Plate."
7. A summary in briefest possible form should close the article.
8. Provide a running page title (not over 35 letters and spaces).

Photographs, preferably supplied unmounted, must be trimmed and arranged to conserve vertical space. Graphs or line drawings should be on white board or blue-line paper. All lettering must be done in black ink and be suitably proportioned to print legibly. Photographs of graphs and drawings that are of inconvenient size for mailing will ordinarily be suitable. Colored photographs are published at the author's expense.

For each manuscript, authors will be allowed the equivalent of $25.00 for the reproduction of illustrative material and will be allowed one page of tabular material without charge. Important additional matter of this sort may be allowed at cost at the discretion of the Managing Editor. The cost of making excessive alterations in Galley proof will be charged to the author.

CORRESPONDENCE

Manuscripts, correspondence relating thereto and new books for review should be addressed to ENDOCRINOLOGY, Editorial Office, *25 Shattuck Street, Boston 15, Massachusetts.*

Correspondence relating to membership applications and Society business should be sent to Dr. Henry H. Turner, *1200 North Walker St., Oklahoma City, Oklahoma.*

All other business matters, including correspondence and remittances relating to subscriptions, back volumes and advertising, orders and remittances for reprints and membership dues should be sent to CHARLES C THOMAS, PUBLISHER, *301-327 East Lawrence Avenue, Springfield, Illinois.*

Entered as second-class matter at the Post Office at Springfield, Illinois, and at Menasha, Wisconsin, under the act of March 3, 1879. Accepted for mailing at special rate of postage provided for in the Act of February 28, 1925 embodied in Paragraph 4, Section 538, P.L. & R.

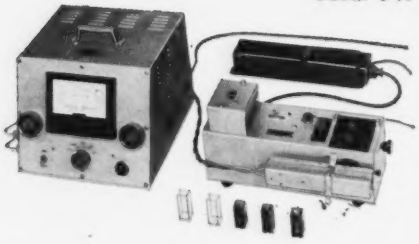
Copyright 1957, by The Endocrine Society. Printed in U.S.A.

PHOTOVOLT

Line-Operated Multiplier

FLUORESCENCE METER

Mod. 540



- High-sensitivity for measurement of low concentrations (full-scale setting for 0.001 microgram quinine sulphate)
- Micro-fluorimetry with liquid volumes as low as 1 ml
- Low blank readings, strict linearity of instrument response
- Interference filters for high specificity of results and for determining spectral distribution of the fluorescent light.

Write for Bulletin No. 392 to

PHOTOVOLT CORP.

95 Madison Ave. New York 16, N.Y.

Specializing

ENDOCRINE ASSAYS

17-Ketosteroids

Beta and Androgen Fractions

Protein-Bound Iodine

Gonadotropins

Pregnanetriol

Pregnanediol

Corticoids

Estrogens

CONSERVATIVE FEES

Write for fee schedule for endocrine and other determinations.

Biochemical Procedures

8350 WILSHIRE BOULEVARD, BEVERLY HILLS, CALIFORNIA

Mail this form to Charles C Thomas · Publisher · Springfield · Illinois and you will receive your copy immediately.

TRANSACTIONS OF THE AMERICAN GOITER ASSOCIATION—1956

•

Many communications have been received indicating that this meeting was an outstanding one, particularly because of the excellence of the papers, dealing with biochemistry and its allied sciences. An internist interprets the results of the various laboratory procedures in terms designating in what manner they might be of practical value.

Complete the form below and we will send you one copy of Transactions of the American Goiter Association ($16.75, 488 pages, 182 illustrations), for ten days free inspection approval.

•

Name .....

Address .....

City .....Zone .....

State .....

A few copies of the 1948, 1949, 1950, 1951, 1952, 1953, 1954, and 1955 Transactions are still available on the same terms.

•

Check the issues you want sent:

- ☐ 1948—320 pp., 100 illus., $6.50
- ☐ 1949—476 pp., 160 illus., $10.50
- ☐ 1950—455 pp., 198 illus., $11.50
- ☐ 1951—525 pp., 222 illus., $12.50
- ☐ 1952—446 pp., 159 illus., $12.50
- ☐ 1953—440 pp., 145 illus., $13.50
- ☐ 1954—496 pp., 52 illus., $14.00
- ☐ 1955—482 pp., 196 illus., $14.00
- ☐ 1956—488 pp., 182 illus., $16.75

Announcing the First Title in the John Alexander Monograph Series . . .

THE SURGICAL MANAGEMENT OF PULMONARY TUBERCULOSIS

Edited by
JOHN D. STEELE, M.D.

Introduction by
FREDERICK A. COLLER, M.D.

Biographical Sketch of John Alexander
By
CAMERON HAIGHT, M.D.

Written for both surgeons and internists by thoracic surgeons who were trained by the late John Alexander and by two of his close medical associates.

COVERS ALL PHASES OF PROCEDURES CURRENTLY USED:

- Indications
- Techniques
- Results
- Standard and plombage thoracoplasty
- Treatment of tuberculous empyema
- Cavernostomy

John C. Jones (Los Angeles) writes on **pulmonary resection for tuberculosis in the pre-chemotherapy era,** including his own experiences in the 1930's; Max Chamberlain (New York) on **segmental resection;** Herbert Sloan and Roger Milnes (Ann Arbor) on the **indications, contraindications, and results of resection.** Problems and methods of the reduction in the size of the pleural cavity with pulmonary resection are discussed in a chapter on combined collapse and resection.

Some of the historical aspects of the surgery of pulmonary tuberculosis have been drawn from John Alexander's books published in 1925 and 1937 and from the uncompleted manuscript for the third book on which he was working at the time of his death. A short chapter on the surgical management of tuberculosis in the mentally ill has been included since this is an important subject and little discussed.

Since **chemotherapy** plays such an indispensable role in the medical and surgical treatment of pulmonary tuberculosis, a comprehensive chapter (which is actually a short monograph) on the subject has been included.

This chapter by Winthrop N. Davey (Ann Arbor) and John B. Barnwell (Washington, D.C.) contains historical material as well as **practical suggestions for modern treatment.** The topics covered include details on antimicrobial agents (both the antibiotics and synthetics), the selection of patients, the selection of drugs, a review of the results and limitations of drug therapy, and the use of chemotherapy in extrapulmonary tuberculosis.

Publication date: May 15, 1957 **248 pages (7" x 10")**
175 illustrations **Cloth, $9.50**

THE JOHN ALEXANDER MONOGRAPH SERIES on Various Phases of Thoracic Surgery

Editor
JOHN D. STEELE, M.D.
San Fernando, California

Editorial Board

Howard T. Barkley, M.D.
Houston, Texas

Cameron Haight, M.D.
Ann Arbor, Michigan

Hiram T. Langston, M.D.
Chicago, Illinois

Herbert C. Maier, M.D.
New York City

John W. Strieder, M.D.
Brookline, Massachusetts

DISTINGUISHED CONTRIBUTORS TO THE FIRST VOLUME

Howard T. Barkley
John B. Barnwell
J. Maxwell Chamberlain
John S. Chambers
Noble O. Correll, Jr.
Winthrop N. Davey
John C. Jones
Hiram T. Langston
Roger Milnes
Paul V. O'Rourke
Clyde E. Rush
Herbert Sloan
George N. J. Sommer, Jr.
John D. Steele
John W. Strieder

CHARLES C THOMAS • PUBLISHER • SPRINGFIELD • ILLINOIS

Estrogen-androgen therapy effectively prevents postpartum breast engorgement

Satisfactory results were obtained in over 96 per cent of cases in a series of 267 patients who received estrogen and androgen as combined in "Premarin" with Methyltestosterone. Therapy was started as soon as possible after delivery. No untoward side effects were noted. In addition, the absence of mental depression in the puerperium was considered of notable importance.*

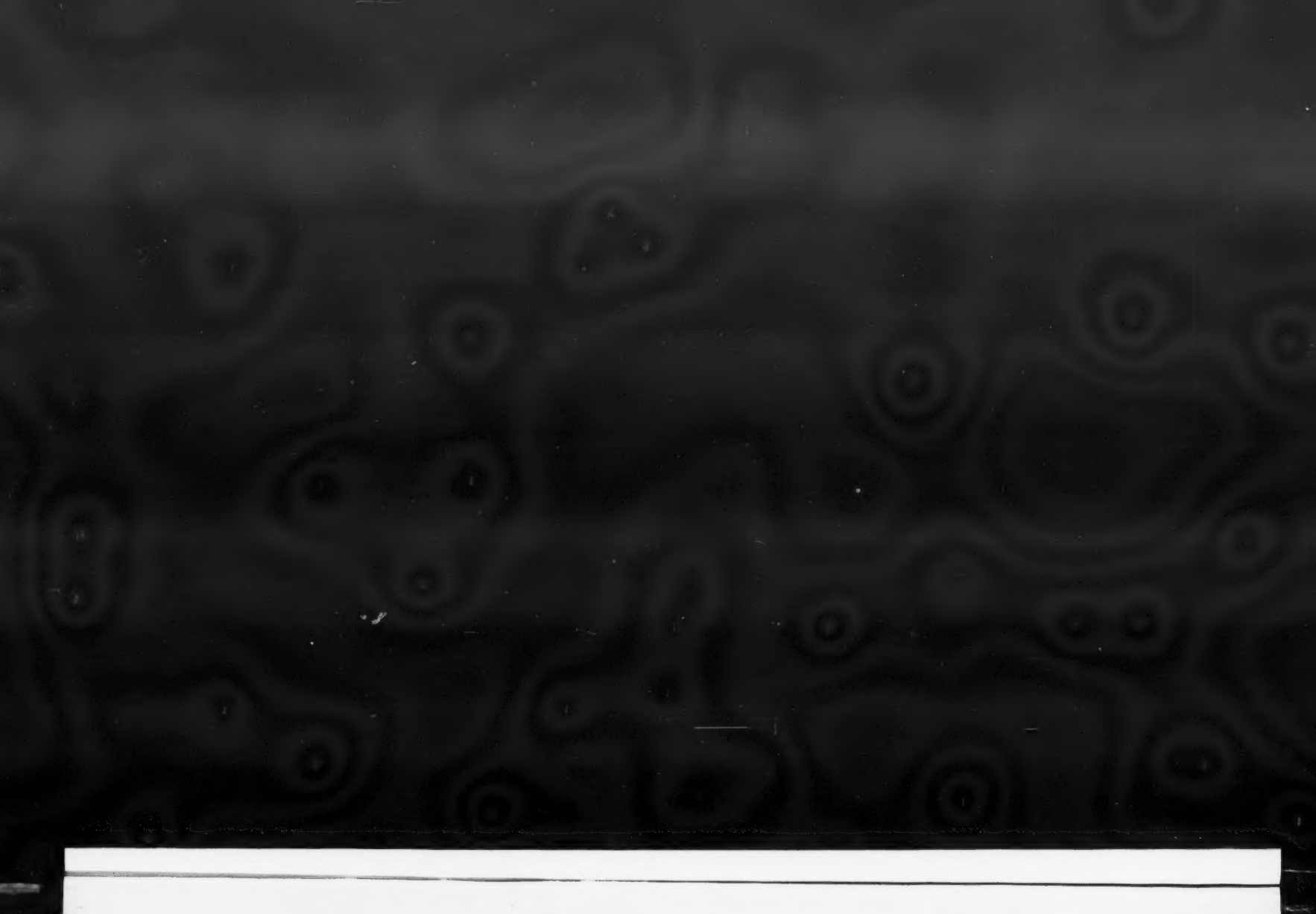
*Fiskio, P. W.: GP *11*:70 (May) 1955.

"PREMARIN"® with METHYLTESTOSTERONE

for combined estrogen-androgen therapy

Ayerst

Ayerst Laboratories • New York, N. Y. • Montreal, Canada

8548

6157